

NEURONAL ENCODING OF BRIEF TIME INTERVALS IN THE VISUAL SYSTEM

by

Joseph Patrick Mayo

B.A., Vassar College, 2001

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This dissertation was presented

by

Joseph Patrick Mayo

It was defended on

March 15, 2011

and approved by

Aaron Batista, Ph.D., Dept. of Bioengineering

Julie Fiez, Ph.D., Dept. of Psychology

Neeraj Gandhi, Ph.D., Dept. of Otolaryngology

James Mazer, Ph.D., Dept. of Neurobiology, Yale University

Daniel Simons, Ph.D., Dept. of Neurobiology

Dissertation Advisor: Marc Sommer, Ph.D. Dept. of Neuroscience

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We see the world as it unfolds in both space and time. Neuroscience research so far, however, has largely focused on the spatial aspects of vision, including orientation and size. No less important to a comprehensive understanding of brain function is an understanding of how visual input is transformed into knowledge about the timing of events in the world. To begin to address this issue, we recorded the activity of single neurons in the frontal eye field (FEF), an area of prefrontal cortex thought to help mediate conscious visual perception. For comparison, we recorded from two portions of the superior colliculus (SC) in the midbrain. The superficial SC receives inputs from the retina and early visual areas, and intermediate SC is associated with early visual processing and the control of eye movements. In two experiments, we measured visual responses to time-varying stimuli and tested whether the magnitudes or latencies of the responses might be used by the brain as a source of timing information.

First, we measured visual responses in individual neurons while two consecutive flashes of light were presented during passive fixation. We found that when stimulus intervals were brief (~200 milliseconds), neurons responded robustly to the first flash but not the second one (“neuronal adaptation”). As intervals lengthened, neurons fired more robustly for the second flash. Thus, information about time was implicit in the size of successive visual responses.

We then asked if this timing information is exploited by the brain. We recorded activity in FEF and SC while monkeys performed a time interval discrimination task. We evaluated the

“magnitude hypothesis”, stemming from our adaptation findings, and the “latency hypothesis”, which predicts that time intervals are encoded by the relative latencies of visual responses. We found that performance in the task was best described by the magnitude hypothesis; larger visual responses were associated with longer passages of time. We conclude that neuronal adaptation may play a functional role in time perception. Thus, the timing of visual events in the world, at short naturalistic timescales, is partly encoded by the magnitude—not just the latency—of neuronal activity.

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PREFACE

Chapter 2 has already been published. The work in Chapter 3 is in preparation:

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1.0 GENERAL INTRODUCTION

1.1 TEMPORAL ASPECTS OF VISUAL PROCESSING

Successful interaction with the world depends on an accurate representation of time. Our sensory and motor systems rely critically on precise neuronal processing at the level of hundreds of milliseconds. Given the primary importance of timing for perception and action, it is surprising that the neurophysiological mechanisms of temporal processing remain largely unexplored (Hemmen and Sejnowski, 2006; Hegdé, 2008).

One of the greatest successes of systems neuroscience has been the classification of brain areas involved in visual processing. The transformation of visual information entering the retina to visual percepts and motor commands has been investigated for decades, yielding fairly comprehensive circuit diagrams (Felleman and Van Essen, 1991; Wallisch and Movshon, 2008). Vision therefore provides an ideal setting for conducting basic research on how stimuli are processed and used to guide behavior.

Investigations of the temporal aspects of vision so far have focused on the early stages of visual processing (e.g., Bair et al., 1994; Albrecht, 1995; Bredfeldt and Ringach, 2002). Precise work on the temporal aspects of visual neurons have been carried out here, in areas critical for vision but not generally thought to underlie our conscious visual experience. In contrast, there has been limited research on temporal processing in prefrontal cortex, especially at the level of

single neurons (Genovesio et al., 2006; Li and Lisberger, 2011). Prefrontal cortex is often assumed to be the locus of higher cognitive processing (Miller, 2000), but it is still unclear how neurons in prefrontal cortex help maintain an accurate sense of the time of visual events.

1.2 PSYCHOPHYSICS

Our ability to discriminate time intervals is remarkably labile (for review, see Eagleman, 2008). Human time perception can be influenced by basic, low-level changes in visual stimuli. For example, in certain situations simply changing stimulus identity can lead to dramatic distortions in time perception (Rose and Summers, 1995). Top-down effects such as spatial attention can also bias time perception (Yeshurun and Levy, 2003; Rolke et al., 2007; van Boxtel et al., 2010). Furthermore, eye movements themselves can bias time perception, leading to time illusions either just before or just after a saccade is made (Yarrow et al., 2001; Morrone et al., 2005; Georg and Lappe, 2007). Given the frequency with which we allocate our attention to different items in the world by shifting our gaze, it is remarkable that we are able to track occluded objects or time our movements to high-five at a sporting event.

Although careful experimentation by psychophysicists and experimental psychologists has revealed the limits of time perception, there has been considerably less progress made in understanding the neural basis of time perception, and timekeeping in general. The most popular idea from experimental psychology is the Scalar Expectancy Theory (SET) and the related pacemaker-accumulator model (Gibbon, 1977; Church, 1984). This ubiquitous model posits a central “internal clock” mechanism that regularly emits timed pulses. These pulses are then accumulated (by unknown areas and mechanisms) and compared with a reference duration stored

in memory. The result of this comparison is read out linearly in a process that corresponds to conscious time perception. More pulse accumulation leads to longer perceived intervals and less accumulation leads to shorter intervals. This model and variations of it have been invoked since the early 1970's to account for many aspects of time perception (Cabeza de Vaca et al., 1994; Church et al., 1994; Kanai and Watanabe, 2006), and it is still popular today (e.g., Klink et al., 2011).

Although the pacemaker accumulator model provides a mechanistic framework, it has produced few insights into the neural underpinnings of time perception. Without a more rigorous biological foundation, SET's utility may be limited. We propose starting from physiological evidence and building piecemeal on our existing knowledge of visual processing. The goal of this approach is to understand time perception as a cognitive process comparable to other topics in systems neuroscience such as object recognition, visual search, and attentional allocation.

An overarching aim of this dissertation is to begin to provide a neurophysiological framework for understanding time perception at the level of single neurons.

1.3 NEURONAL ADAPTATION

Given the multitude of ways in which time perception can be distorted—and the many timescales on which it functions—it seems likely that time perception, like other sensory-driven cognitive processes, is tied to the neuronal mechanisms that process sensory stimuli. One putative timing mechanism is what we call “neuronal adaptation”, also known as repetition suppression (for review, see Kohn, 2007). Adaptation occurs when stimuli are presented in quick succession, as

commonly happens during natural behavior. Neurons do not show identical responses to all stimuli in a sequence. Instead, after the first response, subsequent responses are diminished (Fig. 1, top). In the visual system, the time interval between stimuli required for adaptation is around half of a second, depending on the brain region and stimulus parameters. Critically, the size of subsequent responses depends on the amount of time since the preceding response; the shorter the interval, the smaller the response. All visual areas tested so far show this diminishment of activity at shorter time intervals (V1: Judge et al., 1980b; IT: Brown et al., 1987; SC: Robinson and Kertzman, 1995; MT: Lisberger and Movshon, 1999; V4: Motter, 2006; LIP: Lehky and Sereno, 2007; FEF: Chapter 2, Mayo and Sommer, 2008). Although prolonged (seconds and longer) adaptation of neuronal activity occurs in many brain regions (e.g., Kohn and Movshon, 2003; Solomon et al., 2004), we use “neuronal adaptation” here to refer specifically to brief (tens to hundreds of milliseconds) response changes that are induced by the presentation of a single visual stimulus.

The precise physiological mechanisms of adaptation are unclear. Adaptation may be caused in part by biophysical fatigue, in the form of synaptic depression and vesicle depletion. Repeated firing likely leads to a lack of neurotransmitter release during the time required for refilling. This may account for the temporal aspect of adaptation because faster rates of stimulation lead to smaller responses via less time for recovery from fatigue. Repeated stimulation at the same frequency therefore results in a balance between fatigue and firing. This balance is seen in neuronal recordings as a lower plateau of responses (Fig. 1, top), not as increasingly diminished activity (Fig. 1, bottom). Also, there is evidence that adaptation leads to a prolonged hyperpolarization after the initial sensory response (Carandini and Ferster, 1997). These cellular mechanisms likely interact with local-circuit dynamics. Specifically, differences

in excitatory and inhibitory influences on pools of neurons can theoretically account for the changes seen during adaptation (Heeger, 1992). Given the variety of physiological factors thought to influence adaptation (for review, see Clifford and Rhodes, 2005), it is not surprising that visual areas differ in terms of their adaptation dynamics. For the purposes of this dissertation, the central point is that adaptation is time-dependent and ubiquitous, and therefore seems to be a promising candidate mechanism for keeping track of time.

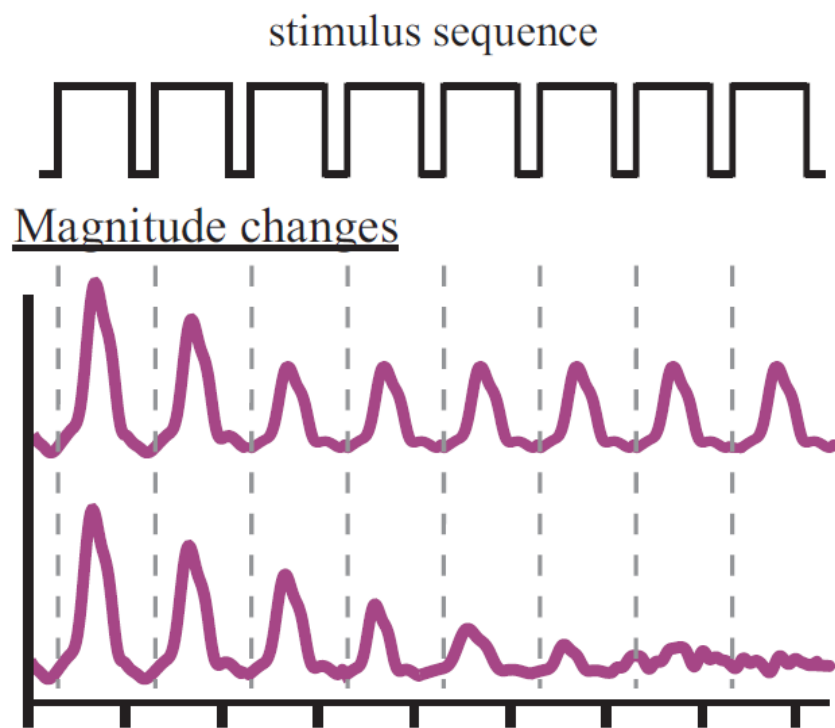


Figure 1. Neuronal adaptation

Schematic of hypothetical changes in response magnitude when stimuli are repeated at a constant rate. *Top*, representation of adaptation as it occurs in the visual system. Activity decreases to a plateau then remains constant. *Bottom*, alternative scenario in which adaptation drives responses to near zero over time. (Modified from Mayo, 2007).

1.4 LATENCY AND MAGNITUDE ENCODING OF TIME

A visual stimulus occurring at time X leads to a wave of neuronal activity in the brain's circuitry occurring at time X plus a certain neuronal latency, Y . $X+Y$ is then roughly the time the stimulus is experienced. At the long timescales commonly used in the laboratory setting, this "latency hypothesis" is incontrovertible; a flash of light presented to the eye today causes neuronal responses and perception today. There can never be a complete dissociation of neuronal latency and time perception.

At short timescales, however, the association between neuronal latency and perception is less obvious. Natural vision is carried out at the level of hundreds of milliseconds (ms) because primates move their eyes approximately three times per second (Henderson and Hollingworth, 1998; DiCarlo and Maunsell, 2000). During each of these saccadic eye movements, visual input to the brain is effectively turned off while the eyes are in motion (Reppas et al., 2002; Kleiser et al., 2004; Ibbotson et al., 2008). This dampening of visual processing during eye movements, called saccadic suppression (Matin, 1974), prevents us from seeing the world as smeared as it rapidly travels across our retina. The sub-second time intervals between saccades, when the eyes are still, are therefore the natural time windows for visual analysis. At this naturalistic timescale, does the latency hypothesis still hold true?

The latency hypothesis may not hold true for sub-second intervals for two reasons. First, at finer timescales, spikes from adjoining visual responses are compressed in time. Responses can partially or completely overlap one another, potentially making it difficult to disambiguate the beginning and end of consecutive responses. This compression is less of a problem in temporally-precise sensory modalities like the auditory system, which can represent sounds with single millisecond precision (Tzounopoulos and Kraus, 2009; Kayser et al., 2010). But the

visual system is slower, trading temporal precision for the detailed richness of our visual experience. The inherent variability in the latency and transmission delays of neuronal responses (e.g., Gur et al., 1997; Nijhawan, 2008) therefore may become problematic at shorter time scales. Variability of, say, 10-20 ms in the timing of the neuronal response onset to a stimulus will likely not impact the encoding of a 3-second interval between successive stimuli, but may have a profound influence on the encoding of a 150-ms interval. Furthermore, visual responses to stimuli separated by less than a second are subject to duration-dependent changes due to neuronal adaptation. Because of adaptation, successive visual responses consist of fewer spikes, following the first response in the sequence.

Given the ubiquitous nature of neuronal adaptation and its implicit magnitude code for time intervals, it is possible that changes in magnitude between two consecutive visual responses may be used for perception (“magnitude hypothesis”). The inherent magnitude code could offer a complement to latency coding when latencies are noisy or ambiguous. In this way, sub-second interval encoding may depend on both the latency and magnitude of neuronal responses.

Does the magnitude or latency hypothesis more accurately describe the neuronal encoding of natural time intervals? What happens to the size and timing of responses when durations are misperceived? Do brain areas at different levels of the visual hierarchy differ in their ability to represent perceived time? The experiments in this dissertation are designed to answer these questions.

1.5 FRONTAL EYE FIELD AND SUPERIOR COLLICULUS

We evaluated the magnitude and latency hypotheses by recording from two structures with similar response properties but which are positioned at contrasting ends of the visual hierarchy: the superior colliculus (SC) in the midbrain and the frontal eye field (FEF) in prefrontal cortex (Fig. 2). Neurons in both regions exhibit a continuum of visual- and eye movement-related responses (Mohler et al., 1973; Bruce and Goldberg, 1985). The two structures are reciprocally linked via mono- and di-synaptic connections (Segraves and Goldberg, 1987; Stanton et al., 1988a; Helminski and Segraves, 2003; Sommer and Wurtz, 2004). Although anatomically simple, the FEF-SC circuit has a profound influence on visual perception and our ability to orient to objects of interest (Schiller et al., 1974; Chafee and Goldman-Rakic, 1998).

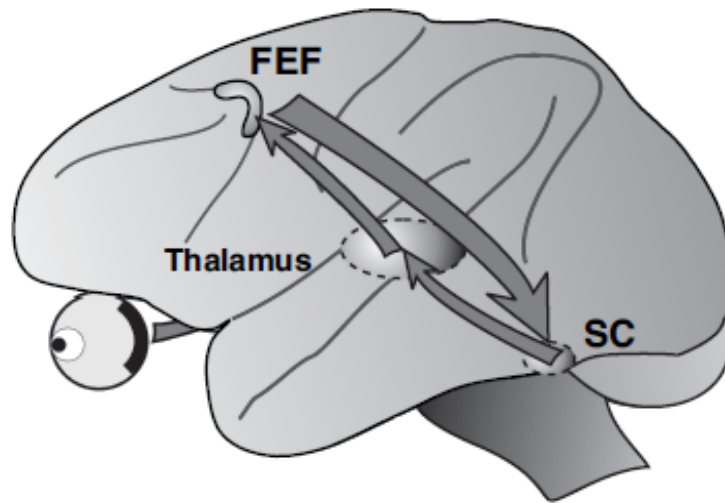


Figure 2. Schematic illustration of SC-FEF visuomotor pathway

Visual information enters the eye and is sent to the thalamus and midbrain SC for additional processing

FEF: Located on the anterior bank of the arcuate sulcus in prefrontal cortex, FEF has been implicated in higher cognitive functions such as spatial attention (Moore and Armstrong, 2003), visual analysis (Bruce and Goldberg, 1985; Schall, 2002), and conscious visual perception (Thompson and Schall, 1999; Gold and Shadlen, 2000; Miller and Cohen, 2001; Libedinsky and Livingstone, 2011). Inactivation and lesion studies support these reported functions (Sommer and Tehovnik, 1997; Dias and Segraves, 1999; Schiller and Chou, 2000).

FEF may play an important role in representing time intervals for two reasons. First, FEF receives disynaptic input from both the basal ganglia and the cerebellum (Fig. 3), two structures that have long been thought to play critical roles in temporal processing (Spencer et al., 2003; Mauk and Buonomano, 2004; Jahanshahi et al., 2006; O'Reilly et al., 2008). Second, FEF—as a locus of eye movement control and perception—is an obvious candidate area for influencing time perception via changes in sensory processing (e.g., saccade-induced time illusions). Therefore the anatomical and functional properties of FEF make it an ideal candidate for investigating the sensory influences of visual time perception.

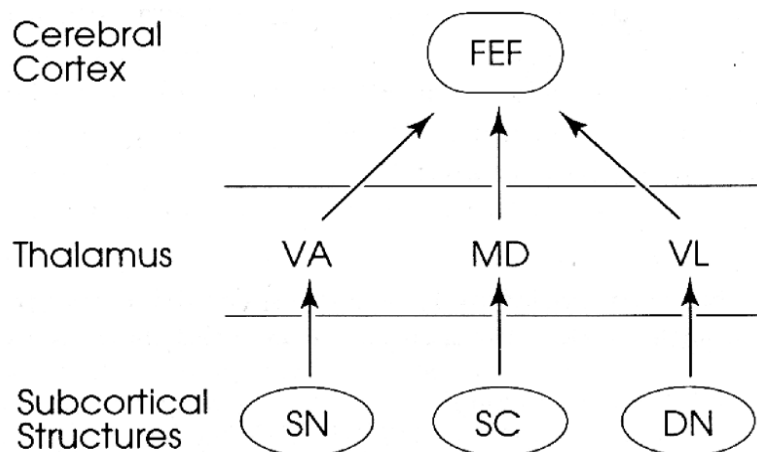


Figure 3. Diagram of anatomical inputs FEF

Connections from the basal ganglia (substantia nigra; SN) and cerebellum (dentate nucleus; DN) to FEF (top) via nuclei in the thalamus (Lynch et al., 1994).

SC: Located along the roof of the midbrain, the SC is distinguished by its seven layers of cells and fibers. These layers are divided into superficial (I-III; supSC) and intermediate and deep layers (IV-VII; intSC). The superficial layers are visually responsive and receive monosynaptic input from the retina (Astruc, 1971; Hubel et al., 1975; Fries, 1984). Evidence from cats and rabbits suggests that supSC may also receive input from primary visual cortex (Bereshpolova et al., 2006). Superficial SC neurons show no eye movement-related activity and can be distinguished extracellularly from neurons in deeper layers by their brisk firing and densely packed cell bodies (Cynader and Berman, 1972; Richmond and Wurtz, 1980). Activity from supSC is sent out mainly through nuclei of the thalamus to areas of visual cortex. Neurons in the intermediate layers of SC show a range of visuomotor responses (Wurtz and Goldberg, 1971; Mohler and Wurtz, 1976; Walker et al., 1995). Responses in deep SC (not recorded in this dissertation) show a similar range of responses, although motor responses are generally more prominent (Sparks, 1988; Ma et al., 1991). In accordance with the reported responses of single neurons, SC has been shown to be a vital component in the generation of eye movements and in visual orientation (Schiller and Koerner, 1971; Wurtz and Goldberg, 1971).

1.6 EXPERIMENTAL AIMS

A primary goal of this dissertation is to understand the changes in FEF and SC neuronal activity that may govern visual time perception. We have two specific aims:

Aim 1. Measure visual responses to consecutive stimuli in FEF

To understand conscious time perception, we need to first measure neuronal responses in prefrontal cortex to stimuli presented at behaviorally relevant time scales. In Chapter 2 we compare the amount of adaptation in FEF during passive fixation with established rates in SC. The goal of Chapter 2 is to quantify “baseline” measures of neural activity and determine the appropriate temporal parameters to use for more sophisticated studies of temporal discrimination.

Aim 2. Correlate changes in single neuron activity with performance in a time discrimination task

The magnitude hypothesis posits that time intervals are encoded in the relative strengths of successive visual responses. The latency hypothesis predicts that intervals are encoded in the relative latencies of successive responses. Both hypotheses make strong predictions about performance in a time discrimination task. If the magnitude hypothesis is true, errors in time perception should be associated with deviations of second response magnitudes. If the latency hypothesis is true, errors should be associated with deviations of second response latencies. In Chapter 3 we test these hypotheses by recording visual responses in FEF and SC while monkeys report their trial-by-trial judgments in a time interval discrimination task.

2.0 NEURONAL ADAPTATION CAUSED BY SEQUENTIAL VISUAL STIMULATION IN THE FRONTAL EYE FIELD

2.1 ABSTRACT

Images on the retina can change drastically in only a few milliseconds. A robust description of visual temporal processing is therefore necessary to understand visual analysis in the real world. To this end, we investigated sub-second visual changes and asked how prefrontal neurons in monkeys respond to stimuli presented in quick succession. We recorded the visual responses of single neurons in the frontal eye field (FEF), a prefrontal area polysynaptically removed from the retina that is involved with higher level cognition. For comparison, we also recorded from small groups of neurons in the superficial superior colliculus (supSC), an area that receives direct retinal input. Two sequential flashes of light at varying interstimulus intervals were presented in a neuron's receptive field. We found pervasive neuronal adaptation in FEF and supSC. Visual responses to the second stimulus were diminished for up to half a second after the first stimulus presentation. Adaptation required a similar amount of time to return to full responsiveness in both structures, but there was significantly more neuronal adaptation overall in FEF. Adaptation was not affected by saccades, although visual responses to single stimuli were transiently suppressed postsaccadically. Our FEF and supSC results systematically document sub-second

visual adaptation in prefrontal cortex and demonstrate that this adaptation is comparable to, but stronger than, adaptation found earlier in the visual system.

2.2 INTRODUCTION

The frontal eye field (FEF) in prefrontal cortex has been implicated in visual analysis, cognitive processing, and saccade generation (Bruce and Goldberg 1985; Bruce et al. 1985; Schall 2002). These functions require precise spatial and temporal organization. Whereas a long history of research has investigated the spatial properties of FEF neurons (Mohler et al., 1973; Bruce and Goldberg, 1985; Umeno and Goldberg, 1997, 2001), much less is known about the temporal properties of FEF visual responses. In other visual areas, presenting two visual stimuli in quick succession has a profound effect on neuronal activity; the response to the second stimulus is severely diminished. We term this phenomenon "neuronal adaptation". Such adaptation has been found in many parts of the brain (IT: Baylis and Rolls 1987; Brown et al. 1987; V1: Judge et al. 1980b; V4: Motter 2006; LGN: Schiller 1968; for review, see Kohn, 2007), but has never been characterized in FEF.

Understanding the temporal aspects of FEF neuronal activity is critical for three reasons. First, in order to provide insight into the neuronal basis of cognition, experiments on cognitive processing in FEF may benefit from utilizing two or more visual stimuli flashed in quick succession (e.g., Thompson and Schall, 1999). Neuronal adaptation could influence the results of such studies. The extent to which FEF neurons are susceptible to adaptation, however, is unknown.

Second, understanding sub-second temporal processing is important for determining how the visual system processes natural visual scenes. Many real world changes (e.g., visibility of prey or the actions of a predator) occur at sub-second timescales that are outside the temporal boundaries of most previous electrophysiological studies, especially those performed in frontal cortex. Thus, it is crucial to establish the fundamental temporal characteristics of FEF visual activity at a fast, ecologically-relevant timescale.

Lastly, the study of visual timing is critical for understanding how movements influence perception. It has been shown that temporal judgments about visual stimuli change around the time of a saccade. Specifically, perceived durations shorten presaccadically and lengthen postsaccadically (Matin et al., 1972; Yarrow et al., 2001; Morrone et al., 2005; Georg and Lappe, 2007). As an interface between vision and eye movements (Schall, 2002), FEF seems well-positioned to play a central role in such temporal effects.

The first step in understanding temporal influences on visual processing in FEF is to study neuronal adaptation caused by two sequentially presented stimuli. We asked whether adaptation occurs for FEF neurons, and if so, how it is affected by interstimulus interval and saccade generation. As a point of comparison, and in order to verify our methods, we additionally studied adaptation in neurons in the retinal-recipient superficial layers of the superior colliculus (supSC), an important visuosaccadic structure located in the midbrain. Neuronal adaptation in SC has been well-characterized in previous work (Dorris et al. 2002; Fecteau and Munoz 2005; Robinson and Kertzman 1995; Wurtz et al. 1980). We predicted that the direct retinal input to supSC, as compared to the absence of such input to FEF, would lead to relatively reduced levels of neuronal adaptation in the subcortical structure.

We found that neuronal adaptation in both FEF and supSC is strong for interstimulus intervals (ISIs) less than 100 milliseconds, weaker for relatively longer ISIs, and absent for ISIs greater than 400 milliseconds. Neurons in FEF showed significantly more adaptation than neurons in supSC. For both FEF and supSC, we found no difference in adaptation immediately after a saccade, as compared to during steady fixation, despite clear suppression of the visual response itself postsaccadically. Our results illustrate differences in duration-dependent visual responses at contrasting levels of visual processing. Furthermore, they provide a foundation for carrying out future studies in FEF using visual stimuli presented at real-world time scales.

2.3 METHODS

Two probes were presented consecutively (i.e., temporally nonoverlapping) in the RF of a neuron while the animal fixated. The probes were identical but appeared at slightly different locations near the RF center to minimize afterimages and lower-level adaptation effects (Movshon and Lennie, 1979; Marlin et al., 1991). We varied the amount of time between the two probes and included single probe control conditions to help quantify the temporal dynamics of neuronal activity in response to repeated visual stimulation. We developed the metric “penetrance” to quantify the extent to which a visual response was evoked by the second visual stimulus of a successive pair of stimuli.

2.3.1 Animals and surgery

Four rhesus monkeys (*Maccaca mulatta*; monkeys C, K, M, and N) were anesthetized and surgically prepared for neuronal recordings and eye position measurements. Using aseptic procedures, titanium screws and an acrylic implant were affixed to the skull. Recording chambers and a head-restraint socket (Crist Instruments, Hagerstown, MD) were embedded in the implant. Chambers were positioned over FEF (Monkeys C, K, and N) and supSC (Monkeys K and M) using stereotaxic coordinates (FEF: A23 and L20; SC: angled 40 deg. posterior from vertical, centered mediolaterally on the midline, and aimed at a location 1 mm posterior and 15 mm above stereotaxic zero). In the same surgery we implanted scleral search coils (Judge et al., 1980a). Animals recovered for at least 1-2 weeks before training resumed. All experimental procedures were approved by and conducted under the auspices of the University of Pittsburgh Institutional Animal Care and Use Committee and were in compliance with the guidelines set forth in the United States Public Health Service Guide for the Care and Use of Laboratory Animals.

2.3.2 Behavior

We trained monkeys to perform a simple visually-guided saccade task to targets, while other visual stimuli (“probes”) were flashed in the receptive field (RF; Fig. 4). The probes were irrelevant to the task demands for reward. A head-restrained monkey viewed visual stimuli that were projected onto a tangent screen. Every trial began with the presentation of a fixation spot (Fig. 4, cross) in the center of the screen. After foveating the spot, the monkey was required to

maintain fixation for 1000-2000 ms within a $2 \times 2^\circ$ invisible window. During this presaccadic period, probes might be flashed in the RF as discussed below (“PRE” probes in Fig. 4). A saccade target was then displayed outside of the neuron’s RF, and the monkey had 500 ms to make a saccade to the target (window: $\sim 3 \times 3^\circ$; saccade period in Fig. 4). A second, postsaccadic period of fixation then occurred at this new location (1000-2000 ms). This was a second period during which probes might appear in the RF (“POST” probes in Fig. 4). Finally, to keep the task as similar as possible in both fixation periods, we had the monkey make another saccade to end the trial (not shown); after the second fixation period elapsed, a second saccade target appeared and the monkey made a saccade to it (window: $\sim 3 \times 3^\circ$) within 500 ms. Maintenance of fixation in this window for 200-500 ms completed the trial and resulted in a liquid reward. Breaking fixation, making saccades outside the target windows, or generating late saccades resulted in an aborted trial and no reward. Saccade target positions remained identical within a block of trials and aborted trials were re-run later in the block.

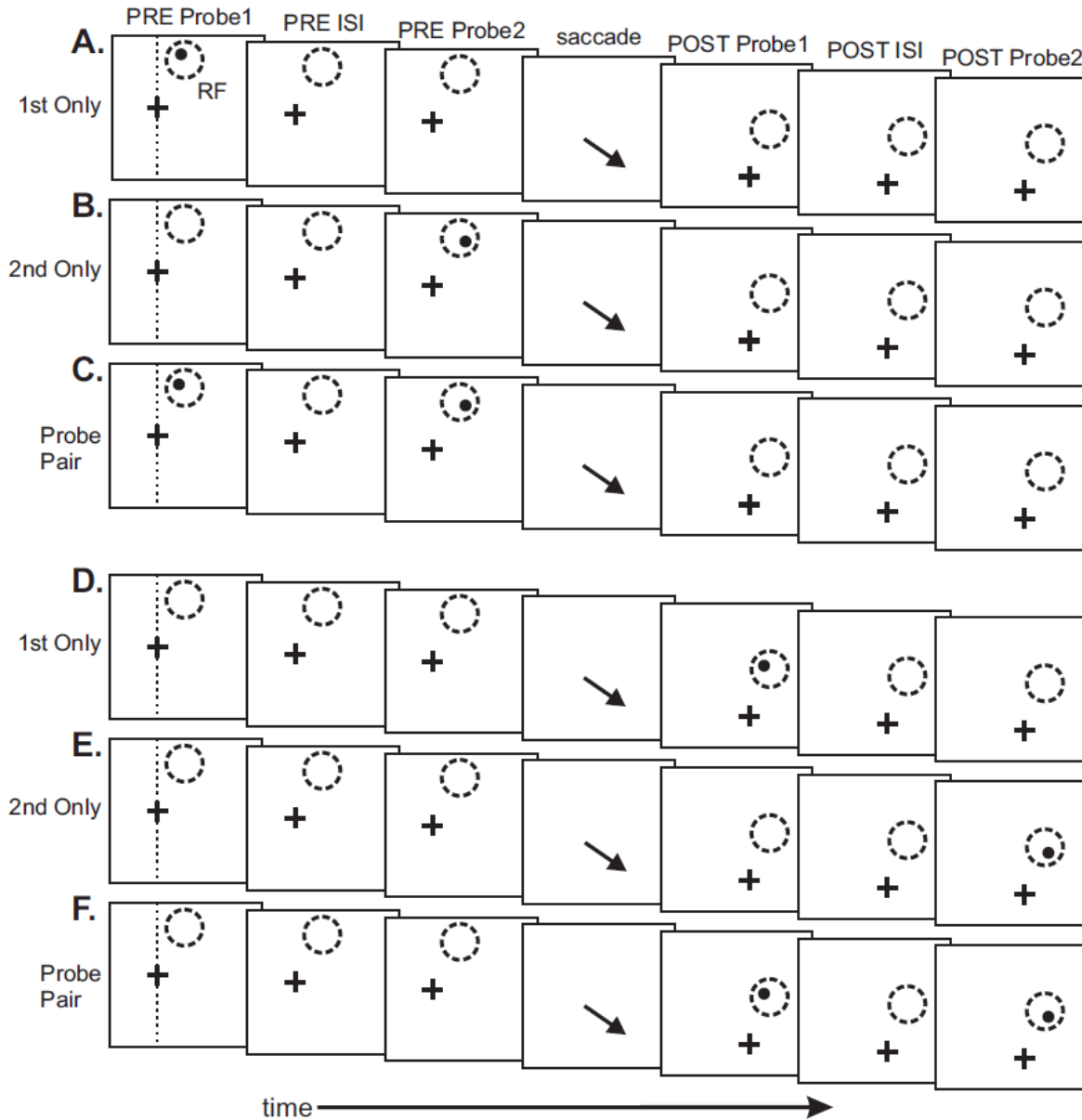


Figure 4. Layout of the adaptation task

Task progresses from start (left) to finish (right). Vertical dotted line represents the vertical meridian of the visual field and dashed circle represents the neuron's RF (neither was visible to the monkey). Each trial began with an initial period of fixation at the center of the screen (cross; leftmost three columns) which was followed by a saccade (arrow; middle column) and a second period of fixation (rightmost three columns). The trial ended with a second saccade (not shown). Visual probes were presented before the saccade in PRE trials (A-C) and after the saccade in POST trials (D-F). 1st Only (A and D) and 2nd Only (B and E) control conditions used a single probe at times identical to those of the Probe Pair condition (C and F). Probes were never presented both in the PRE and POST periods in the same trial. For clarity of illustration, all stimuli are shown as black, but see text for colors used in the experiment. ISI, interstimulus interval, ranged from 17-800 ms.

Randomized Parameters

Two key parameters were randomized on a trial-by-trial basis: the number of probes presented (one or two) and when they appeared relative to the saccade (before or after).

Number of probes: 1st Only, 2nd Only, and Probe Pair trials

Neuronal adaptation is elicited by flashing two probes sequentially and measuring the response to the second probe relative to that of the first probe. Thus, to accurately quantify the adaptation, it is important to measure the response to each probe when presented individually. We measured visual responses to individual probes using first probe only trials (“1st Only”; Fig. 4A, D) and second probe only trials (“2nd Only”; Fig. 4B, E). In the test condition for eliciting adaptation, the same two probes appeared sequentially; these were “Probe Pair” trials (Fig. 4C, F).

Probe presentation relative to the saccade: Presaccadic vs. postsaccadic

In half of the trials, probes were presented before the saccade (termed “PRE” trials; Fig. 4, A-C). In the other half, probes were presented after the saccade (“POST” trials; Fig. 4, D-F). The probe timings were the same in PRE and POST trials except for when the first probe appeared. The PRE trial period began with an initial foveating saccade of random direction and amplitude. To minimize any potential influence of these widely varying saccades on the subsequent visual responses, we delayed onset of the first probe for 200 ms after start of fixation. In contrast, the POST trial period followed a well-controlled, stereotyped saccade (Fig. 4, middle column). Here we could more carefully test saccadic influences on visual responses. For POST trials, we timed the first probe to start 30 ms after start of fixation.

For both PRE and POST trials, the onset time of the second probe, when present (i.e., in Probe Pair and 2nd Only conditions), varied according to the particular ISI for a given block (see next paragraph). We timed the events so that in both the PRE and POST conditions, the second probe appeared long before a saccade (200-600 ms prior to its onset) to minimize the effects of saccade preparation on our visual responses. The main point of using the two conditions was to determine if adaptation differed immediately after a saccade (POST condition) in comparison to before a saccade (PRE condition). As will be reported at the start of the Results section, we found no significant differences in the majority of our data between PRE and POST trials, so for most analyses PRE and POST data were pooled.

Manipulation of ISI

The ISI was kept constant within a block of trials and randomly changed between blocks. ISI refers specifically to the amount of time between the offset of the first probe and the onset of the second probe. Six ISIs were examined: 17, 50, 100, 200, 400, and 800 ms. It was not feasible to run the entire range of ISIs for each neuron. On average, we collected full data sets (i.e., all combinations of PRE/POST and 1st Only, 2nd Only, and Probe Pair conditions) for two to four ISIs in each recording session.

Spatial configuration of the task

Probes were white (40 cd/m^2 , $0.6 \times 0.6^\circ$) and one frame ($\sim 17 \text{ ms}$) in duration presented on a dark background (0.8 cd/m^2). The fixation point was blue (1.2 cd/m^2 ; $0.3 \times 0.3^\circ$) and saccade targets were red ($0.3 \times 0.3^\circ$). To minimize afterimages or low-level adaptation effects (Movshon and Lennie, 1979; Marlin et al., 1991), we did not present the two probes at the same location in a

given Probe Pair trial. Instead, we gave the probes a small spatial offset. For FEF recordings, probes were spatially offset 4° from each other, symmetrically around the center of the RF; each probe was 2° away from the RF center. For supSC recordings, to account for the smaller diameter RFs, probes were 1° away from the RF center (Cynader and Berman, 1972; Wurtz et al., 1980). The axis of the probe offset locations was always orthogonal to the fovea-RF axis to minimize apparent radial motion effects (Xiao et al., 2006).

It is crucial to note that the location of the first presented probe in a pair was randomized between the two offset locations and balanced so that each probe appeared in the first ordinal position for an equal number of trials. Hence if there were any slight differences in visual response or adaptive influence between the two probe locations, our results represented the average. However, quantitative analyses revealed few if any differences. As reported in Results, the two probe locations nearly always elicited identical visual responses.

In all trials, saccades were directed outside of the RF to eliminate any saccade related activity, which is common in FEF neurons (Bruce and Goldberg, 1985). RFs and saccade target positions were located contralateral to the recording hemisphere or along the vertical meridian.

2.3.3 Recording and data collection

At the start of a recording session, a single tungsten electrode (300k-1MΩ impedance @ 1 kHz; FHC Inc., Bowdoinham, ME) was lowered through a guide tube (23 gauge) using a custom microdrive system (LSR, 2008; details at <ftp://lsr-ftp.nei.nih.gov/lsr/StepperDrive/>). A plastic grid with 1 x 1 mm hole spacing (Crist et al., 1988; Crist Instruments, Hagerstown, MD) was placed inside the recording chamber to map the topography of the underlying structures. FEF,

located along the anterior bank of the arcuate sulcus, was identified as the region in which fixed-vector saccades were evoked more than 50% of the time using low-current ($<50 \mu\text{A}$) microstimulation (Bruce and Goldberg, 1985). Superficial SC was identified physiologically by neurons with brisk visual activity and small, topographically arrayed RFs located about 1 mm above neurons with broader visuomotor responses in the same structure (Richmond and Wurtz, 1980). The characteristic topographic maps for both structures were confirmed with systematic recording and stimulation mapping (Robinson, 1972; Bruce et al., 1985). Standard extracellular recording techniques were used to isolate action potentials from single neurons (FEF; Sommer and Wurtz, 2000). Electrode stability for recording multiunit activity (supSC) was implemented via continuous online waveform inspection.

Once a neuron was isolated, its RF was mapped using an iterative procedure that located the center of the RF in a precise but time-efficient manner. The mapping procedure involved having the monkey make saccades to visual targets at a variety of spatial locations. Receptive fields in both FEF and supSC are, at least roughly, topographically organized across the physical surface of the structures (Robinson, 1972; Bruce et al., 1985). Thus, the response field location changed only gradually (if at all) during a penetration, so any time we encountered a neuron/multiunit site we already had a general idea about where its response field should be located. To begin mapping a RF for a neuron, we initially presented a “direction series” of stimuli. A visual stimulus would appear in one of 8 directions, spaced by 45 degree increments around the clock (the cardinal and diagonal directions), at the presumed best amplitude. This series revealed the best direction for the neuron; it could be in any of the 8 directions, or if the response was equally maximal for two adjacent directions, the best direction would be considered in the middle of those two adjacent directions. Hence our resolution in terms of

direction was initially 22.5 degrees. Then we ran an “amplitude series” in which a visual stimulus appeared at 1 of 8 eccentricities at the best direction to find the best amplitude for the neuron (1-35°). To be thorough, we then re-ran the direction series using the same eight directions but now centered on the confirmed best amplitude (rather than the presumed best amplitude as done on the first pass). We continued this process of iterating between the direction and amplitude series (Sommer and Wurtz, 2004) until we were certain that we had found the best direction and amplitude of the neuron (the center of its response field). The physical limits of our display, along with the required spatial configuration of the task, precluded us from using neurons with highly eccentric RFs (centers greater than ~30° peripheral). During the mapping procedure, neuronal activity was monitored online using real-time raster plots and histograms.

To characterize the separate visual and movement activity of each neuron, we placed a target at the center of the mapped RF and had the monkey make memory-guided saccades to it (Mays and Sparks, 1980; Hikosaka and Wurtz, 1983). The memory delay period was 500-1000 ms, randomized in 100 ms increments (see Sommer and Wurtz, 2000 for full details). After mapping the RF and confirming a visual response, we began evaluating the neuron while the monkey performed the main experimental task (as described above; Fig. 4).

Behavioral paradigms and data collection were controlled using the REX real-time system (Hays et al., 1982). Neuronal activity and eye position (Riverbend Instruments, Birmingham, AL) were sampled at 1 kHz and time stamps of each action potential were saved offline for further analysis. On average, we collected 15 trials (minimum of 10) for each stimulus arrangement per ISI condition for each neuron. Saccades were detected online using velocity criteria and verified offline using template matching procedures. Saccade start- and endpoints were confirmed by the investigator for each trial during analysis.

To provide visual stimulation, the REX system controlled a separate computer with its own graphics card. Once the latter computer was commanded to present a pair of visual probes, it independently timed the ISI with no further input from REX, thereby providing precise probe timings. The stimuli were back-projected by an LCD projector (ViewSonic PJ550, Walnut, CA) onto a tangent screen (Stewart Filmscreen, Torrance, CA) in a dimly lit room. The screen was positioned ~58 cm from the monkey's eyes and extended ~43° x 38° of horizontal and vertical visual angle, respectively. Screen refreshes and exact stimulus onset and offset times were detected continuously online by a photocell circuit. Photocell data were recorded by the behavioral computer and used to correct for the lag as a function of vertical location caused by the rastering of the projector.

2.3.4 Data analysis

For offline data analysis, spike trains were transformed to spike density functions (SDFs) by convolution with 3 ms wide Gaussians (MacPherson and Aldridge, 1979). We also tried Gaussians 1-10 ms in width but found that 3 ms best characterized the qualitative effects observed in the raw data. All analyses and figures use 3 ms SDFs unless noted otherwise.

Penetrance

To characterize changes in neuronal activity, our main goal was to measure the first neuronal response and its effect on the strength of the second response. We defined the term “penetrance” as a measure of neuronal adaptation. Penetrance quantifies the extent to which a neuron responds to a second probe when it is part of a pair, compared with when it is presented alone

(i.e., not preceded by a first probe). In other words, penetrance represents whether flashing two visual probes in a row results in a second visual response that is obliterated (0% penetrance), moderately potent (0-99% penetrance), or as strong as a normal single visual response (100% penetrance). For the sake of clarity, we chose to refer to the amount of *remaining* activity rather than the amount of *missing* activity of the second neuronal response. In essence, penetrance is the inverse of “suppression”, which quantifies the extent to which a visual response is squelched. To our knowledge there is no alternative term in physiology that succinctly describes the magnitude of neuronal activity that remains detectable (or “penetrates”) despite the effects of adaptation.

The first step in calculating penetrance was to measure the magnitude of the response to a second probe when it was presented as part of the Probe Pair ($Response2_{Pair}$) compared with the response when it was presented in the 2nd Only condition ($Response2_{Alone}$). An initial “uncorrected” measure of penetrance was the difference in areas under the $Response2_{Pair}$ and $Response2_{Alone}$ SDFs. Specifically, we calculated the $Response2_{Pair}$ SDF by subtracting the activity contributed by the first probe, as measured in the 1st Only condition, from the activity evoked by the Probe Pair (Fig. 5A, left). A byproduct of this calculation was that it removed baseline activity. The remaining activity represented the response to the second probe of a pair, i.e. $Response2_{Pair}$ (Fig. 5B, left). We then transformed the visual response to the 2nd Only probe (Fig. 5A, right) into a comparable function, $Response2_{Alone}$, by subtracting baseline activity from it (Fig. 5B, right). Baseline activity was measured during initial fixation in a 50 ms window centered on the onset of the first probe.

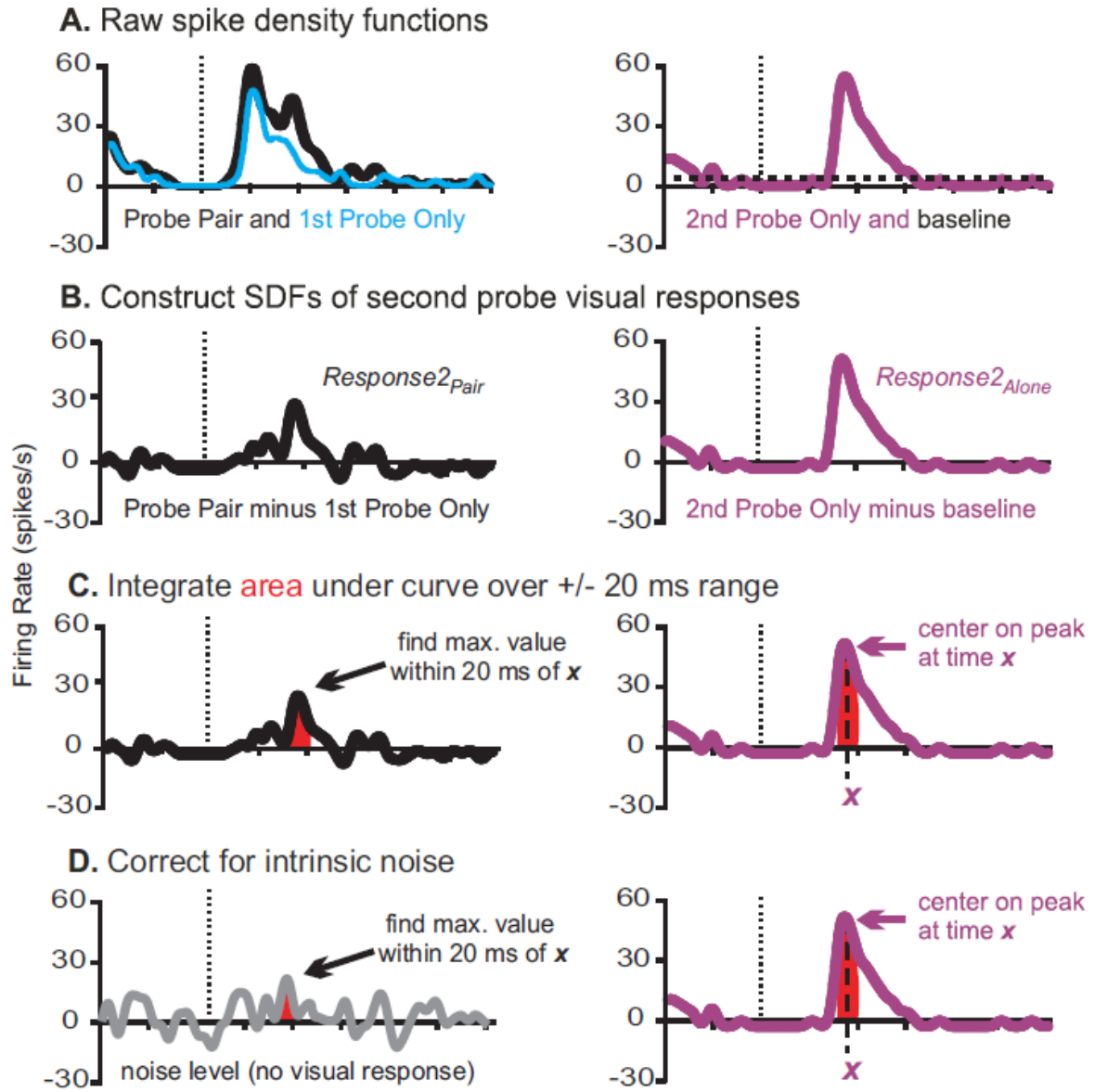


Figure 5. Illustration of steps used to calculate “penetrance”

A, (left): Probe Pair (black) and 1st Probe Only (blue) SDFs. (Right): 2nd Probe Only SDF (purple) and baseline activity (black dashed line). Gray vertical dashed lines indicate time of first probe onset. B, (left): $Response2_{Pair}$ SDF obtained by subtracting the 1st Probe Only SDF from the Probe Pair SDF. (Right): $Response2_{Alone}$ SDF obtained by subtracting baseline activity from the 2nd Probe Only SDF. C, Integration of area (red) in the response epoch of (left) the $Response2_{Pair}$ SDF and (right) the $Response2_{Alone}$ SDF. See text for details. D, Intrinsic noise SDF (gray, left) used to determine background penetrance relative to the full $Response2_{Alone}$ epoch (right). Each tick mark on the abscissa represents 100 ms.

The next step was to quantify the difference between the visual responses in the *Response2_{Pair}* and *Response2_{Alone}* SDFs (Fig. 5B, left and right, respectively). The visual response was measured as the area under the SDF within a 10 ms epoch (shorter and longer epochs were tried as well, with little difference in results). The visual response in the *Response2_{Alone}* SDF was always clearly identifiable, so we centered the epoch at the peak of this response (Fig. 5C, right, time x). In contrast, the visual response in the *Response2_{Pair}* data was not as easily identifiable because of the neuronal adaptation effect. One simple way to address the response ambiguity would be to center the *Response2_{Pair}* epoch at the same time as the *Response2_{Alone}* epoch, i.e. at time x . However, we were concerned that the *Response2_{Pair}* visual response could be slightly offset in latency from the *Response2_{Alone}* visual response. That is, it seemed possible that a preceding stimulus could affect visual latency as well as magnitude. To compensate for potential shifts in latency, we varied the *Response2_{Pair}* epoch relative to the *Response2_{Alone}* epoch (over $x \pm 20$ ms) until the area under the *Response2_{Pair}* visual response was maximal (Fig 5C, left). Next, we subtracted the two areas (*Response2_{Alone}* visual response - *Response2_{Pair}* visual response) to yield a differential visual response in units of spikes/s. This value was transformed to a percentage of the single-probe visual response of the neuron under study, so that we could use this normalized value to compare adaptation between neurons that may have widely varying visual responses. (To quantify the single-probe visual response we repeated the above calculations, but compared the 1st Probe Only SDF to a segment of the SDF far from the visual response.) The differential visual response was expressed as a percentage of the single-probe response to yield the “uncorrected penetrance”.

For penetrance to have useful meaning, it should be zero if a visual response were completely absent in the *Response2_{Pair}* curve. However, our algorithm is agnostic as to whether

a visual response occurs in *Response2_{Pair}* and so it necessarily finds the maximum positive area under this SDF over the range $x \pm 20$ ms, regardless of whether it is caused by the second probe or noise. The end result is that “uncorrected penetrance” cannot reach zero. Our next goal was to adjust for this analytical artifact and obtain a full range, 0-100%, penetrance metric. To this end, we found the actual minimum level, or “intrinsic penetrance”, by calculating penetrance values with test curves having identical noise characteristics but known to lack a second visual response. We constructed such curves using the 1st Only trials. For each neuron, half of the 1st Only trials were randomly selected, pooled, and transformed into a SDF. The same procedure was used for the other half of the 1st Only trials. The difference between the two SDFs eliminated the visual response but retained the inherent noise level (Fig. 5D, left). This differential SDF was used as a stand-in for the *Response2_{Pair}* data and was subtracted from the *Response2_{Alone}* data (Fig. 5D, right) to calculate the intrinsic penetrance of the neuron. We found that the average intrinsic penetrance (solely attributable to noise fluctuations) was ~24% for FEF. Using this as the lower bound for uncorrected penetrance values, the values were rescaled to a final corrected value in which 0% represents no visual response. “Penetrance”, as used for the remainder of this paper, will refer to this final corrected value.

Normalization of population data

To account for variations in intensity of visual responses between neurons when compiling the population graphs of Figure 7, we normalized each neuron’s responses to the maximum firing rate in the visual response epoch (50-150 ms after stimulus onset) for the 1st Only PRE condition for each neuron at every ISI. The neuron’s entire SDF was then divided by this maximal response, and visual activity across all neurons for a given ISI was averaged to get the final

normalized population response. For Figures 7B-C, the normalized 1st Only response was subtracted from the normalized Probe Pair response to obtain the residual 2nd probe response traces. The maximal response (Probe Pair responses in Fig. 7A, residual 2nd probe responses in Figs. 7B-C) was normalized to 1.0 and the remaining activity scaled accordingly.

Saccade direction versus penetrance

In order to look for a relation between impending saccade direction and penetrance, we normalized the saccade target positions relative to probe position in polar coordinates. Zero degrees equaled a direct saccade to the probes and 180 degrees represented a saccade in the diametrically opposite direction. Saccade directions from opposite hemispheres were converted to this 0-180 degrees scale. Using this scale, we analyzed whether penetrance varied with saccade direction, as described in Results.

Sigmoid fits

Best-fit lines and three-parameter sigmoid curves (Fig. 9) were determined using SigmaPlot 10.0 software package (Systat Software Inc., San Jose, CA). The preferred model (linear vs. sigmoidal) was determined by calculating the Akaike information criterion (AIC; Sakamoto et al., 1986; Akaike et al., 1998). AIC utilizes the residual sum of squares and adds a penalty for additional parameters to deter overfitting the data. A lower AIC value indicates a more appropriate model.

Constructed multiunit FEF responses

To estimate whether our approach of recording single neurons in FEF and multiple neurons in supSC may have affected the comparative penetrance values between the two areas, we created “virtual” multiple neuron recordings from our single neuron FEF data. We combined FEF responses into multiunit-like groups by randomly assigning individual FEF neurons into groups of two or three. This number of neurons per group matched our estimate of the number of neurons isolated per site in the supSC recordings. FEF group SDFs were combined according to ISI and averaged before recalculating penetrance values.

Postsaccadic suppression index

We qualitatively observed a postsaccadic suppression effect in the visual responses, and we quantified this effect by measuring the number of spikes occurring in 1st Only trials during the visual response epoch (50-150 ms after stimulus onset) for PRE versus POST conditions. The total number of spikes was compared before (PRE) and after (POST) the initial saccade using a suppression index of the form $(FR_{pre} - FR_{post}) / (FR_{pre} + FR_{post})$, where “FR” indicates firing rate during the response epoch. Index values ranged from -1 to 1, corresponding to relatively facilitated and suppressed POST responses, respectively.

Latency of neuronal responses

The onset latency of visual activity was estimated using a running, paired t-test that compared the average firing rate in a ± 15 ms interval fixed and centered at stimulus onset (-15 to 15 for the first probe and ISI ± 15 ms for the second probe, both relative to first probe onset) with average firing rates in a sliding interval of the same duration but advanced in 1 ms intervals. Significant

visual activity was defined as periods of activity where $p < .01$ for at least 10 ms. We designated the first significant point in this period as the neuron's latency.

We confirmed the accuracy of our latency values by comparing them with values obtained using a second, simpler method that used the peaks of neuronal activity as temporal landmarks. We determined the response latency of the first peak in a pair using data from the raw Probe Pair SDF. To determine the latency of the second peak in a pair, we used the Probe Pair minus 1stOnly condition (Response2_{Pair}) SDF. All analyses were conducted in conjunction with visual inspection of histograms and raster plots to ensure accuracy. Latencies determined by the two methods ("t-test" and "peaks") were highly correlated and not significantly different in either FEF or supSC (Rank Sum test, t-test vs. peaks, FEF: $p = 0.982$, supSC: $p = 0.984$; regression of t-test vs. peaks, FEF: $r = 0.994$, $p < 0.001$, supSC: $r = .999$, $p < 0.001$).

2.4 RESULTS

Our central hypothesis was that sequential visual stimuli would result in adaptation of visual responses in FEF neurons. Moreover, because FEF is polysynaptically removed from the retina, we predicted that the adaptation would be stronger than that in the retinally-recipient supSC. We tested these ideas by presenting two consecutive visual stimuli separated by various durations (17-800 ms) and recording the neuronal responses in FEF and supSC. Responses to individual probes (1st Only or 2nd Only) were recorded as controls. We studied 40 single, visually-responsive neurons in FEF (Monkey K: 20, Monkey N: 11, Monkey C: 9) and, for comparison, 14 multiunit sites in supSC (Monkey K: 11, Monkey M: 3).

2.4.1 Decreased second response to Probe Pair

2.4.1.1 Single neuron example

We found that neuronal adaptation is strong in FEF neurons. Data from a single FEF neuron are presented in Figure 6. Flashing a single, brief-duration probe in a neuron's RF (1st Only condition, middle row of Fig. 6) yielded a characteristic increase in the rate of activity—a *visual response*—beginning ~60 ms after stimulus onset. Flashing an identical single probe later in fixation (2nd Only condition, bottom row of Fig. 6) elicited similar visual responses. Hence all of the visual stimuli used as probes elicited robust visual responses when presented singly.

On the other hand, strong visual responses were not always seen for probes presented *doubly*, i.e. in the Probe Pair experimental condition. The top row of Figure 6 illustrates the response of a typical FEF neuron to two sequential probe stimuli presented using three ISIs (50, 100, and 400 ms). Similar to the 1st Only condition (middle row), visual responses to the first probe at all three ISIs were strong. However, the response to the second probe could be diminished. Given the identical spatial and temporal aspects of probe presentation in the single and pair conditions, it was evident that decreased responses to the second probe of a pair were caused by the immediately preceding first probe.

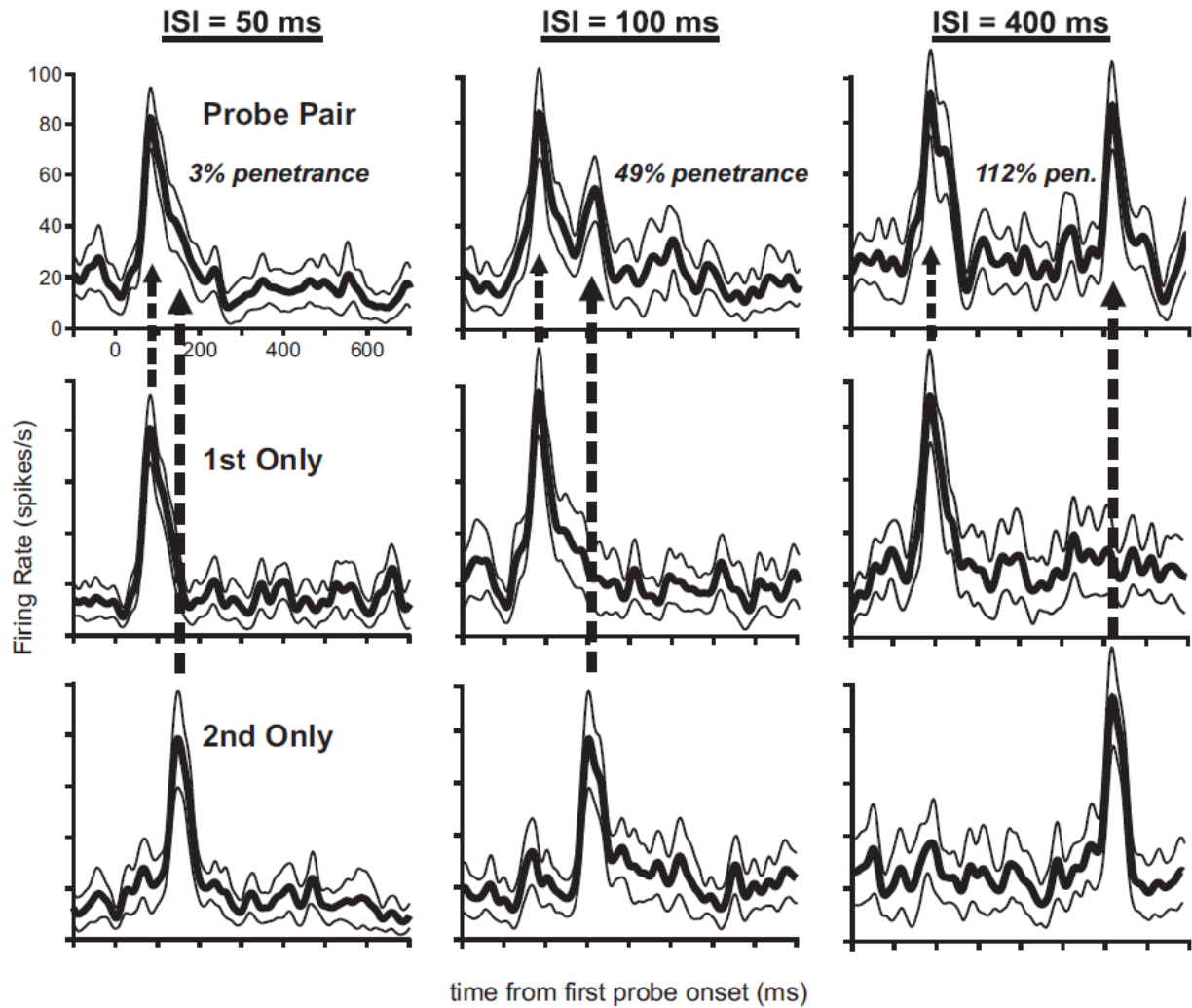


Figure 6. Example visual responses from a single FEF neuron

Top row, Probe Pair condition. Middle row, 1st Only condition. Bottom row, 2nd Only condition. The three columns show results of using ISIs of 50, 100, and 400 ms. Dashed arrows relate timings of corresponding visual responses across rows. Spike density functions depict means (bold lines) and standard error of the mean (thin lines).

At the shortest interval for this neuron (ISI = 50 ms), the presentation of the second probe elicited a negligible visual response (Fig. 6, top-left). At best, the second probe at ISI = 50 ms caused a slight bump in the ongoing response to the first probe. When the time between the probes was increased by 50 ms at ISI = 100 ms, the response to the second stimulus increased to an intermediate level, eliciting roughly half of the typical visual response (Fig. 6, top-center).

Finally, when the Probe Pair stimuli were separated by 400 ms, the neuron displayed two equivalent responses, as if responding independently to each of the single probes (Fig. 6, top-right). These ISI-dependent alterations in visual responsiveness to second probes were quantified using our penetrance metric. For this example neuron, the second probe of the Probe Pair exhibited penetrance levels of 3% at ISI = 50 ms, 49% (about half-recovery) at ISI = 100 ms, and 112% (slightly more than full recovery) at ISI = 400 ms. Thus, the amount of adaptation in the second visual response was a function of time between the first and second visual stimulus.

The example neuron of Figure 6 shows data collected in the PRE condition (see Fig. 4). Data for this neuron in the POST condition were similar. We tested every neuron in both the PRE and POST conditions to compare adaptation effects before and immediately after a saccade. We found no significant differences between ISI-dependent adaptation in the PRE and POST conditions, in either the FEF or the supSC samples (two-way ANOVA on penetrance with ISI and PRE/POST as factors; FEF: $p = 0.259$, supSC: $p = 0.710$). For the rest of this paper we pool the PRE and POST data, unless noted otherwise.

To minimize lower-level adaptation effects (e.g., originating in the retina) we positioned the two probes near the RF center but with a slight spatial offset from each other. One concern was that the two probe locations may have yielded different visual responses. We tested this possibility in a subset of FEF neurons (18 out of 40) and for all supSC multiunit groups. The visual responses were rarely different at the two probe locations (t-test: a single FEF neuron was significant at $p = 0.016$; a single supSC multiunit group was significant at $p = 0.022$). The sequence of probe presentations also did not matter; the results were similar regardless of which probe appeared first (t-test: a single FEF neuron was significant at $p < 0.001$; there were no

significant supSC multiunit groups). As an extra safeguard, we randomized the order of probe presentation so that each of the two probe locations was presented first on half of the trials. Because of this randomization, any differences in visual responses at the spatially offset probe locations could not have influenced our results.

2.4.1.2 Population results

To illustrate the overall adaptation effect in a qualitative manner, we constructed population averages of FEF neuronal activity (Fig. 7A). The firing rate of each neuron was normalized to its maximal response, and the normalized SDFs were averaged for each ISI. As expected, the visual response to the first probe of a Probe Pair was indistinguishable between ISIs (Fig. 7A, arrow). The visual response to the second probe of a Probe Pair, however, increased as ISI increased (Fig. 7A, second visual responses are marked with dots).

We were able to extract the visual response to the second probe at each ISI using the population responses to single and double probe conditions. In Figure 7B, the response to the 1st Only probe was subtracted from the response to the Probe Pair, for each neuron at each ISI, leaving the residual response to the second probe of the pair. This format allows one to appreciate the magnitude of the adapted second probe responses relative to the baseline noise level of activity across the full range of ISIs (17-800 ms).

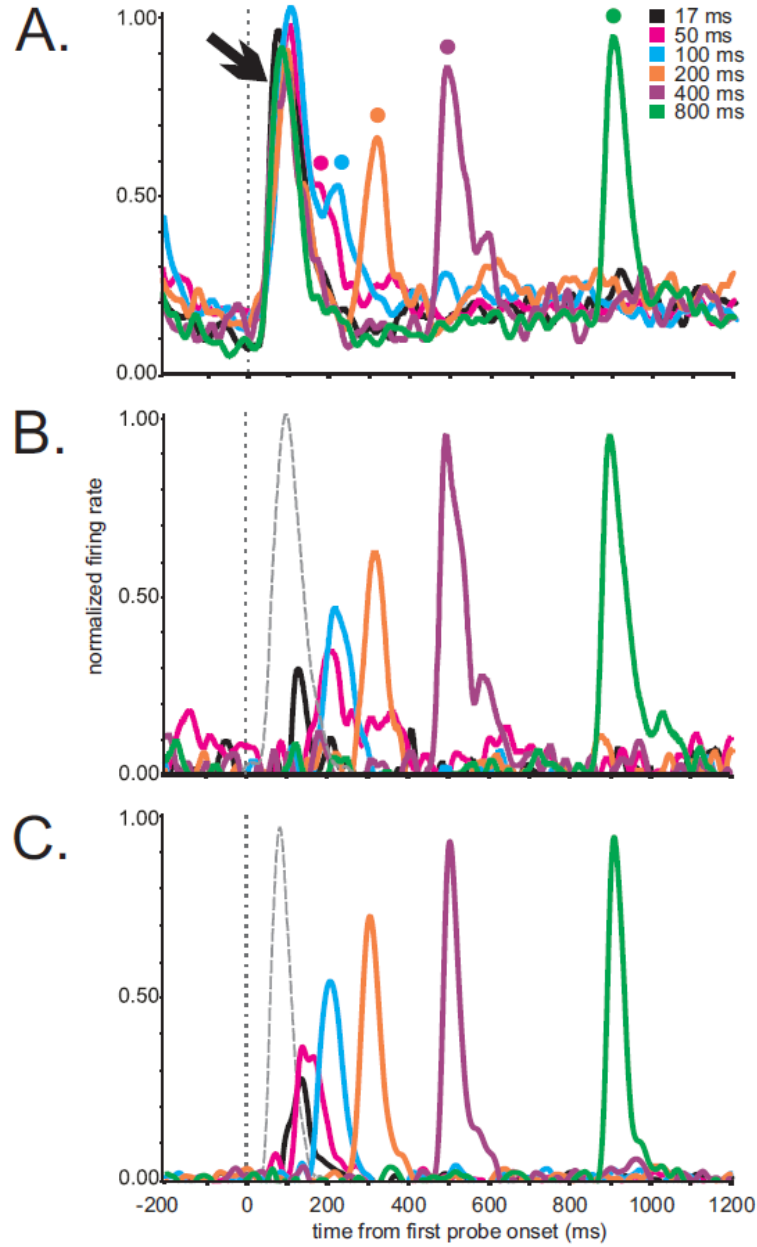


Figure 7. Normalized population responses

A, Normalized visual responses to Probe Pair at each ISI in the FEF population. Vertical dotted line indicates time of first probe onset. Arrow shows main response to first probe; dots label clear responses to second probes. The dot for ISI = 17 ms (not shown) is obscured by the first probe responses. See text for details. **B**, Normalized, baseline-corrected, extracted visual responses to the second probe of Probe Pairs in the FEF population. Responses to first probes, in 1st Only trials, were subtracted from the data in panel A so that only the responses to second probes remained. For reference, the gray dashed curve represents a typical response to a first probe (similar for all ISIs). Spike density functions used 10 ms wide Gaussians for illustration purposes (see Methods). **C**, Superficial SC population responses. Same format as in B.

As Figure 7B illustrates, visual responses to the second probe are discriminable from the noise level even at the shortest ISIs where responses to the first and second probes overlap and absolute responses to the second probe are small. As suggested by the single cell example in Figure 6, in the population activity there is a gradual increase in the response to the second probe with increasing ISI (Fig. 7B). Full visual responses to the second probe were observed at ISI = 400 ms in the population. We also used ISI = 800 ms to ensure that the return to responsiveness was not a temporary reprieve. Responsiveness at 400 and 800 ms ISIs were comparable, suggesting that the time course of adaptation is completely finished after an ISI of 400 ms. The same effect was seen in the supSC population responses (Fig. 7C).

To formally quantify the population data, we calculated the average penetrance at each ISI. For FEF (Fig. 8, black bars), the mean penetrance values as a function of ISI matched what was observed qualitatively in the population responses (Fig. 7B). Penetrance increased with ISI and reached ~100% at 400 and 800 ms. This effect was highly significant (one-way ANOVA, $p < 0.001$). Further analysis revealed that ISI-normalized penetrance did not vary as a function of baseline activity (Pearson correlation; $p = 0.430$), RF eccentricity (Pearson correlation; $p = 0.675$), or the direction of the impending saccade (Pearson correlation; $p = 0.259$; see Methods).

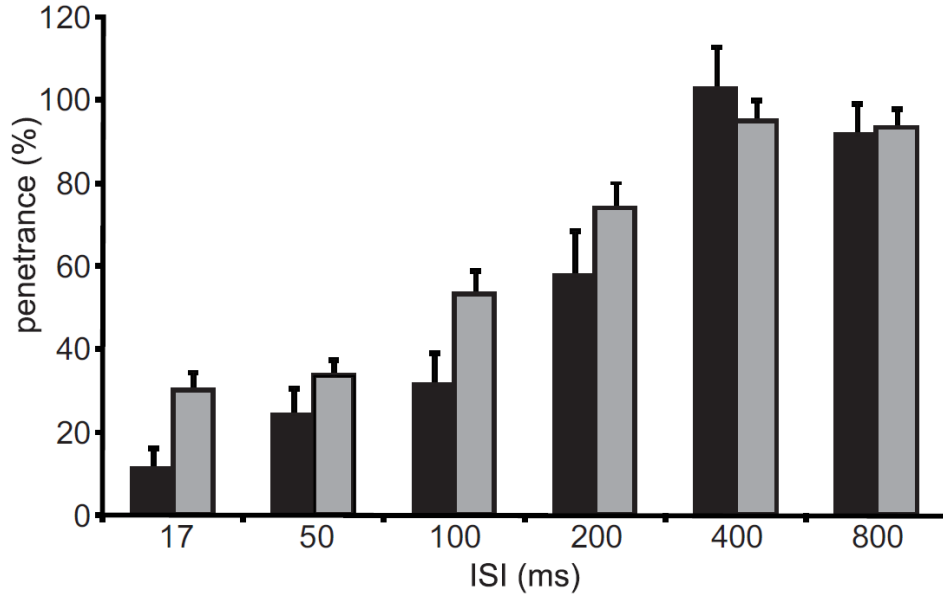


Figure 8. Mean penetrance values with standard error bars for FEF (black) and supSC (gray) populations at each ISI

2.4.2 Comparison of effects in FEF versus supSC

To compare neuronal adaptation at different points along the neuraxis, we recorded neuronal activity in supSC (see Methods) and calculated penetrance values in an identical manner. Previous studies found neuronal adaptation effects in supSC using similar tasks (Wurtz et al., 1980; Robinson and Kertzman, 1995; Dorris et al., 2002; Fecteau et al., 2004). Superficial SC is a midbrain structure located one synapse away from retinal input. Thus it provides an important contrast to FEF in prefrontal cortex which receives no direct retinal input. Because of its close proximity to the retina, one might expect supSC to exhibit less adaptation than FEF.

However, supSC showed the same general adaptation effect as FEF (Fig. 8, gray bars). Like FEF, supSC showed diminished responses at the shortest ISIs but relatively full visual

responses when ISI \geq 400 ms. For our supSC data, as with our FEF data, the change in penetrance as a function of ISI was highly significant (one-way ANOVA, $p < 0.001$).

Neuronal adaptation in both FEF and supSC showed comparable adaptation at corresponding ISIs, but were there significant differences in the effect between the structures? To answer this question, we performed a two-way ANOVA on penetrance using ISI and FEF/supSC as factors. We found a significant main effect of brain area ($p < .001$). The supSC generally showed higher penetrance (less adaptation) at each ISI compared with FEF (Fig. 8).

We next analyzed if the differences between FEF and supSC were due to differences in the *rate* of adaptation (e.g., FEF and supSC showing similar adaptation at short ISIs but differing at long ISIs), or due to differences in *overall* adaptation (e.g., supSC showing uniformly higher adaptation across ISIs). We determined the best-fit linear regression for both the FEF and supSC penetrance values over the range of ISIs where penetrance changed (ISI = 17-200 ms; ISIs of 400 and 800 ms were excluded because their ceiling penetrance values would inappropriately skew the linear regression.) In agreement with the population statistics, we found that the linear regressions were significantly different between FEF and supSC (F-test of regressions; FEF: $y = 0.239x + 5.036$, supSC: $y = 0.252x + 20.267$; $p < 0.001$; Glantz, 2002). This difference in regressions was not due to a difference between slopes ($p = 0.876$), but rather to a highly significant difference between y-intercepts ($p < 0.0001$; Glantz, 2002). The difference cannot be attributed to contrasting noise levels in FEF and supSC because our penetrance metric accounted for such potential inequalities (see Methods). In sum, the rate of neuronal adaptation across ISIs was consistent between FEF and supSC, but the overall level of adaptation differed significantly between the two structures.

To obtain a heuristic description of the difference in adaptation between FEF and supSC, and to facilitate comparisons of adaptation in other brain areas in the future, we calculated the ISI required to elicit 50% penetrance. We determined this ISI value by fitting sigmoid curves to the penetrance vs. ISI data. Over the entire ISI range, sigmoids provided a better fit than linear regression as determined by lower Akaike information criterion values (FEF: AIC = 1090.8 for a sigmoid curve and 1107.3 for a linear fit; supSC: AIC = 622.1 for a sigmoid curve and 654.7 for a linear fit). We plotted penetrance values for all FEF and supSC neurons and fit the resulting scatterplots with three-parameter sigmoid functions (Fig. 9A and B, respectively). The ISI values needed for half penetrance were 163 ms in the FEF and 97 ms in the supSC (Fig. 9C; dashed vertical lines). The separation between the 95% confidence intervals of the data from the two structures at the 50% penetrance level (thin lines, Fig. 9C) indicated that the ISI values at half penetrance (163 ms and 97 ms) were significantly different.

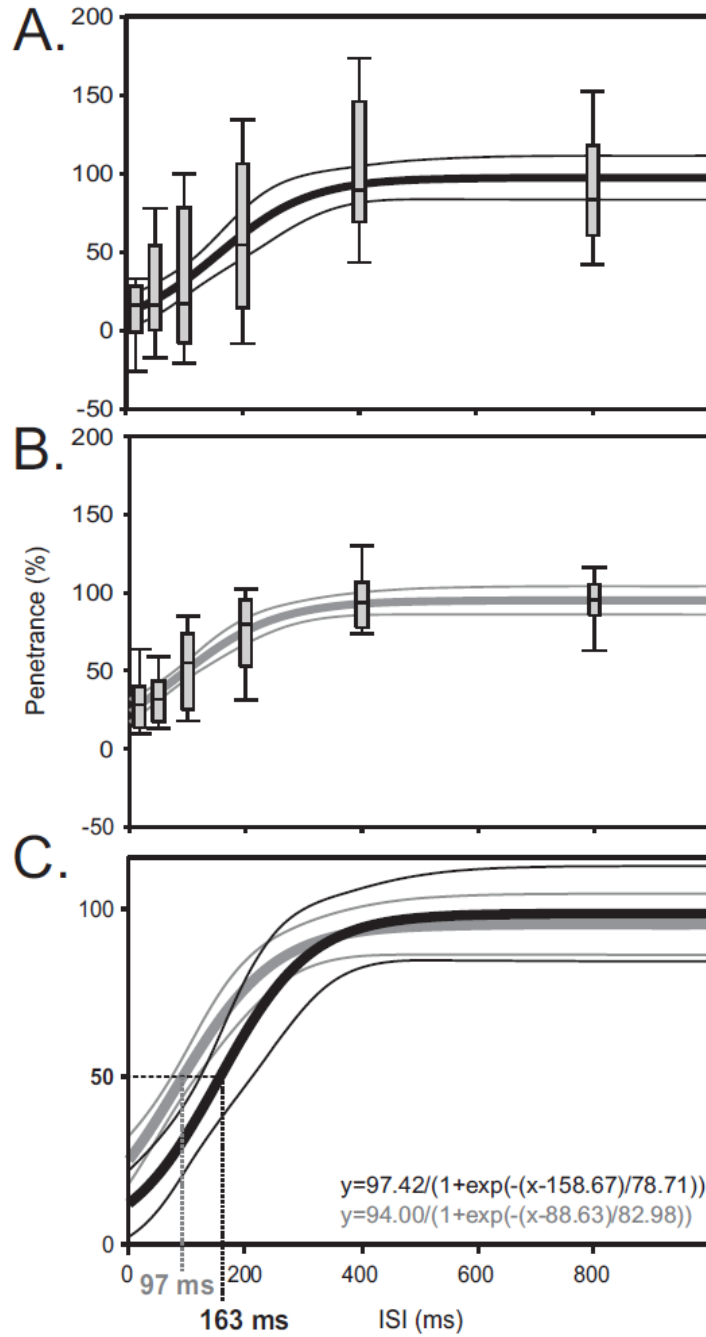


Figure 9. Three-parameter sigmoid fits (thick lines) and box-whisker plots of FEF and supSC data

FEF data in A, supSC data in B. Box-whisker plots show median (line), 25th and 75th percentiles (box), and 10th and 90th percentiles (error bars). Thin lines represent 95% confidence bands. C, Overlay of FEF (black) and supSC (gray) sigmoid fits from panels A and B. Vertical dashed lines indicate the ISI necessary to elicit 50% penetrance. Equations for the sigmoid fits are at lower right.

Despite the nearly identical results between our multiunit supSC recordings and previous single unit experiments (Wurtz et al., 1980; Robinson and Kertzman, 1995), it was possible that the difference in FEF and supSC penetrance values was related to differences in our data collection because we recorded single neurons in FEF but multiple neurons in supSC. We found it difficult to maintain isolation of single supSC neurons for long enough to collect full data files, so we allowed our supSC data to consist of ~2-3 simultaneously recorded single neurons (i.e., multiple single units). It seemed unlikely that this difference would affect penetrance calculations, but to test this hypothesis, we used our FEF data to create post-hoc multiunit responses by randomly placing each single-unit FEF response into groups of 2-3 neurons (see Methods). Combining single neurons into small multiple single unit groups had no effect on the population penetrance values for FEF (two-way ANOVA, $p = 0.808$). Therefore the differences between penetrance in FEF and supSC likely represent true differences in duration-dependent responses between areas rather than artifacts of single versus multiple neuron recordings.

2.4.3 Influence of saccade generation on neuronal adaptation

A wealth of psychophysical research suggests that our perception of time is distorted just before and after an eye movement. Specifically, estimates of temporal duration show a systematic decrease before a saccade and an increase just after a saccade (Matin et al., 1972; Yarrow et al., 2001; Morrone et al., 2005; Georg and Lappe, 2007). It is possible that these perceptual distortions around the time of eye movements are reflected in the corresponding visual activity in FEF. Adaptation changes over time (Fig. 7), and therefore changes in visual response magnitude might provide information about the timing of sequential stimuli. But the perceptual distortions

seen in psychophysical studies suggest that the *latency* of neuronal processing fluctuates perisaccadically. The most straightforward hypothesis is that some mechanism accelerates or delays visual processing in areas underlying conscious vision, such as prefrontal cortex. Such shifts in latency have long been suggested as a possible correlate of spatial attention in psychophysical studies (Titchener, 1908; Stelmach and Herdman, 1991; Carrasco and McElree, 2001), although to date there is relatively little physiological data to support the claim.

We therefore measured the latency of individual FEF neurons' visual responses to Probe Pairs at each ISI (see Methods) and plotted the resulting difference in *response* onset (response onset asynchrony; ROA) and *stimulus* onset (stimulus onset asynchrony; SOA). SOA was used instead of ISI because we could not as easily measure the offset of the first neuronal response, as the neuronal analog of ISI would require. Given the proximity of individual probes to the saccade—the second probe presaccadically and the first probe postsaccadically—we might expect altered ROA values that are inconsistent with the actual Probe Pair intervals. However we found only one significant inconsistent ROA value (ROA = 202 @ SOA of 217 ms; 2-way t-test, $p = 0.047$), and this mismatch was not confirmed using an alternate method that focused on the peak of the visual response (see Methods). In sum, despite the large changes in the adaptation of visual responses with ISI, the *latencies* of visual responses were accurate as a function of ISI. SupSC response latencies were similarly accurate.

To verify this result, we analyzed the influence of an impending saccade on visual responsiveness on a trial-by-trial basis. We ranked individual trials according to saccadic reaction time and normalized the visual responses (see Methods). We tested if the time of an upcoming saccade had a significant effect on presaccadic visual activity. This analysis revealed no significant correlation ($p = 0.167$). This should not be considered surprising, given the

relatively large amount of time between second probe offset and saccade onset, even at the shortest configuration (200-600 ms for ISI = 800 ms).

We did find one clear influence of saccades on our visual responses. It was obvious from inspection of our data that just after a saccade terminated, visual responses were suppressed. To quantify this “postsaccadic suppression” effect, for each neuron we compared the 1st Only visual response in PRE trials (where the stimulus was presented ~200 ms after the initial foveating saccade), with the 1st Only response in POST trials (where the stimulus was presented only ~30 ms after saccade termination). We then calculated a suppression index to quantify the range of suppression in all neurons (see Methods; Fig. 10). We found a significant positive shift in the distribution of index values, indicating relatively diminished visual responses after the saccade in both FEF and supSC (1-sample t-test, null hypothesis = 0; FEF: $p < 0.01$, supSC: $p < 0.05$). The mean index values in both structures were similar (FEF: 0.1281; supSC: 0.110) and the distribution of the two samples did not differ significantly (t-test, $p = 0.804$). Reanalysis of the data after removing any sign of correcting or “catch-up” saccades did not affect the results. It should be noted that this suppression affected visual responses to first probes in both the Probe Pair and 1st Only conditions, so when the latter data were subtracted from the former data in our penetrance analyses, the effect washed out. In sum, our data suggest that saccadic eye movements have a lingering suppressive effect on visual responses that continues at least until ~30 ms after the end of the eye movement.

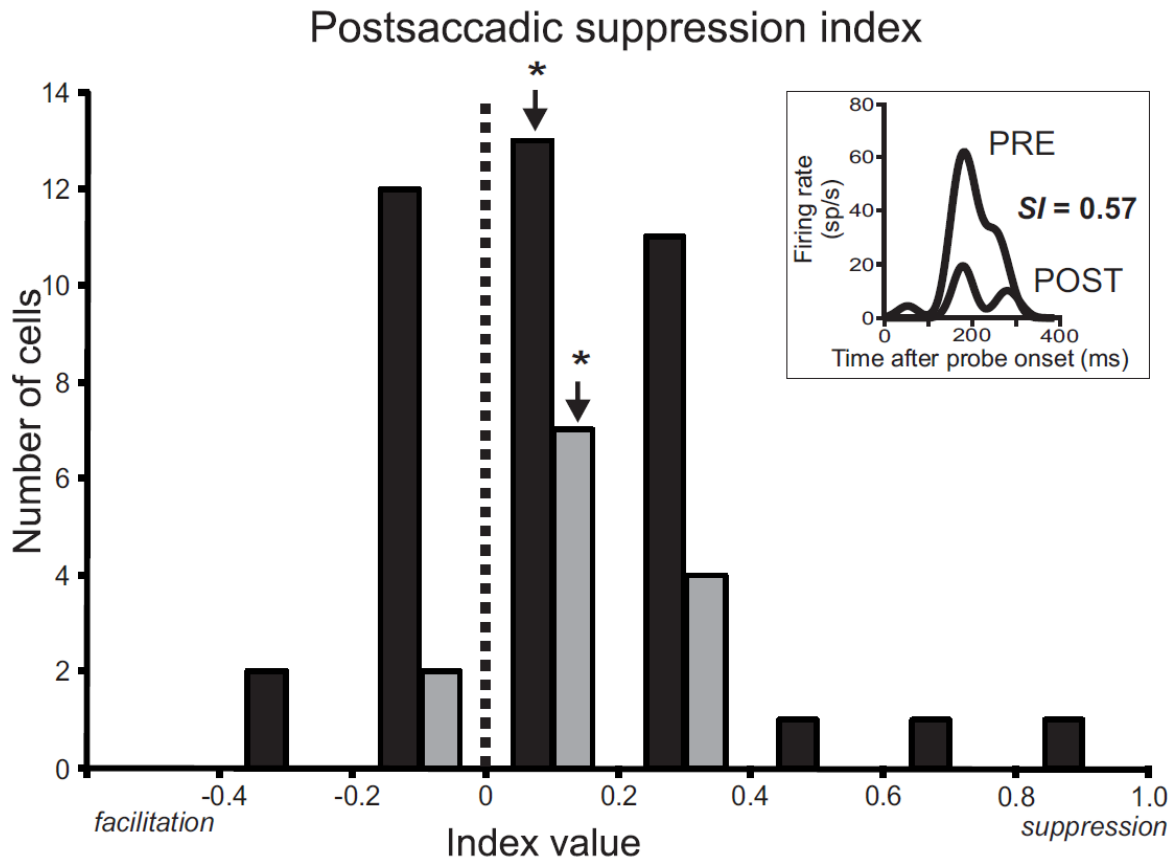


Figure 10. Distribution of postsaccadic suppression index values for FEF and supSC

FEF in black, supSC in gray. Index values were placed into bins 0.2 units wide. Arrows indicate means of the distributions. Stars indicate that the means of the distributions were significantly different from zero. *Inset*, Example FEF visual responses to PRE (larger amplitude) and POST (smaller amplitude) 1st Probe Only conditions, illustrating a neuron with a suppression index value of 0.57. (This particular neuron had a slight biphasic response to the single visual stimulus, especially in the POST condition).

2.5 DISCUSSION

2.5.1 Overview

We characterized neuronal responses to sequential visual stimuli in FEF and, for comparison, supSC. After quantifying neuronal adaptation as a function of ISI, we analyzed differences between the two structures. Finally, we examined the influence of saccade generation on the magnitude and latency of paired visual responses. We found that in FEF the second stimulus of a pair evokes a meager visual response at short ISIs (<100 ms). This response increases with ISI until around 400 ms, where it reaches a normal level. The effect is identical in supSC except for a significant increase in responsiveness across ISIs. The major influence of saccade generation is that, in both structures, visual responses are reduced postsaccadically, confirming the time course of psychophysical results (Diamond et al., 2000).

2.5.2 Adaptation in the visual stream

The present study provides the first systematic analysis of neuronal adaptation in an area of prefrontal cortex, namely FEF. Near the other end of the neuraxis, recordings of ON-responses in the lateral geniculate nucleus (LGN) and optic tract consistently demonstrate a return to full responsiveness at ISIs ≈ 100 -200 ms (Schiller, 1968; Fehmi et al., 1969; Coenen and Eijkman, 1972). This ISI is nearly half of that required for full responses in FEF, suggesting that adaptation in latter areas is not solely attributable to antecedent visual processing. Additionally, the spatial offset of our visual probes precludes a trivial transfer of adaptation entirely from the

earliest visual structures. The two probes of a Probe Pair were close enough in space to activate the RFs of single neurons in FEF, but were likely too far apart to activate the RFs of single retinal, LGN, or V1 neurons (although transfer from, say, V4 or IT is possible). A simple explanation is that FEF contributes to the temporal filtering of visual stimuli. Further experiments are needed to determine how much (if any) FEF adaptation occurs *de novo*, as it appears to do in other visual cortical areas, such as area MT (Priebe et al., 2002).

Surprisingly, comparable temporal studies of neuronal activity in frontal cortex are much less prevalent. Despite the clear importance of understanding the timing of sequential visual responses, relatively few experiments in visual cortex have used simple stimuli at varying intervals and quantified the resulting activity. In V1, Judge et al. showed that two full visual responses can be elicited once flashed stimuli are separated by ~500 ms (1980; but see also Muller et al., 1999 for longer intervals). Recent work in area V4 also suggests a return to full responsiveness at ISIs ~400-500 ms (Motter, 2006). Despite the range of stimuli and procedures used in previous work, our FEF results are generally in accordance with data collected in comparable areas of visual cortex.

Testing supSC neurons as well as FEF neurons provided us with a critical reference point for incorporating our data within the broader context of adaptation studies. Importantly, our results concur with previous research in supSC (Wurtz et al., 1980; Robinson and Kertzman, 1995). Robinson and Kertzman (1995) utilized a task with cue and target presentation in the RF at varying ISIs, similar to our Probe Pair paradigm. In their single neuron data, they found a return to full responsiveness at $ISI \approx 400$ ms, matching our multiunit supSC data. Likewise, Wurtz et al. (1980) presented stimuli in the RF at various ISIs to study visual interactions around the time of eye movements. When the longer duration stimulus was presented first, the mean

percent response (i.e., ~penetrance) at 200 ms—the longest ISI tested—was ~70% in single supSC neurons, nearly identical to our reported value of 74% penetrance at the same ISI. The tight agreement between previous studies and our data suggests that adaptation in supSC is now well characterized and strengthens our confidence in the accuracy of our FEF results.

2.5.3 Neuronal coding and mechanisms

We propose that synaptic short-term plasticity (STP) may be important for the observed adaptation effect. STP involves a transient decrease in synaptic strength based on the frequency of neuronal activity (Zucker, 1989; Fortune and Rose, 2001). STP is governed by calcium-dependent release of neurotransmitter which, in turn, is limited by the rate at which the available pool of calcium replenishes. The initial large response and subsequent diminished response in neuronal adaptation may therefore be explained by the initial release and firing-during-refilling of calcium stores in the neuron. Thus, because adaptation in supSC appears to last nearly twice as long as that of LGN (see above), our results generate the testable hypothesis that recovery from STP is particularly fast in LGN relative to other retinal-recipient visual structures.

Although STP in early visual areas must cause some of the adaptation observed in FEF, it cannot account for all of the FEF adaptation in our study, due to the spatial offsets we used when presenting paired stimuli. The offsets were small relative to the size of a RF in FEF but large relative to the size of a RF in areas such as LGN and striate cortex. In early visual areas, one set of neurons would be activated by the first stimulus of a pair, and a different set of neurons by the second stimulus, so STP-mediated adaptation as described above would seem unlikely. It could be that lateral inhibition in early visual areas (Hartline et al., 1956; Ratliff, 1972) also contributes

to FEF adaptation. But we suggest that STP in FEF itself, and in other extrastriate areas with relatively large RFs, is a more parsimonious explanation. Intracellular work, for example in slices, would likely be needed to test these hypotheses.

One open question is, does adaptation serve a purpose? Neuronal adaptation may function as an efficient means of coding the novelty of incoming visual information (Ringo, 1996; Wiggs and Martin, 1998; Wark et al., 2007). In this schema, repeated stimulation by identical stimuli leads to a “normal” first response followed by interval-dependent diminished responses that decrease to a plateau (Mayo, 2007). Indeed, visual responses in V4 and IT—areas with direct connections to FEF (Schall et al., 1995; Ungerleider et al., 2007)—have demonstrated precisely this phenomenon (Li et al., 1993; Xiang and Brown, 1998; Motter, 2006). However, because FEF neurons are relatively insensitive to stimulus features (Mohler et al., 1973), it is likely that neuronal adaptation in FEF functions more as a general detection mechanism (likely in the service of eye movements) than a specialized form of short-term object memory as found in IT.

2.5.4 Perception and inhibition of return

If spatial attention were automatically drawn to the probes, we would expect to see a firing pattern in line with psychophysical data on inhibition of return (IOR; for review see Klein, 2000). Specifically, we would see a higher firing rate at the shortest ISIs because of increased attentional discriminability (Bushnell et al., 1981; Spitzer et al., 1988). We observed some evidence for this effect because initial visual responses (Fig. 7A, arrow) tended to be larger for shorter ISIs (17-100 ms) due to summation of the first and second visual responses in the raw

data. In this sense, our data are consistent with previous IOR-related SC results (Fig. 7A; Dorris et al., 2002; Fecteau et al., 2004; Fecteau and Munoz, 2005). This slight amplification of raw firing rate notwithstanding, second visual responses at short ISIs were severely adapted, as demonstrated by subtracting the time-matched single-probe visual responses (Fig. 7B).

Our neuronal data also dovetail with a previous study that measured the neuronal correlates of perceptual awareness in FEF (Thompson and Schall, 1999). In that experiment, visual stimuli were presented in the RF at very short ISIs; a target was followed 0-50 ms later by a masking array. Consistent with our data, in that study the first visual response to the target was normal while the second response to the stimulus in the array tended to be weak. Our quantification of neuronal adaptation in FEF could explain the diminished second-stimulus responses seen by Thompson and Schall (1999).

2.5.5 Future directions

By characterizing the “baseline” activity of FEF visual neurons to sequential stimuli, we open an avenue for future research into the effects of eye movements on stimuli presented just before and after the saccade and accompanying temporal illusions (Matin et al., 1972; Yarrow et al., 2001; Morrone et al., 2005; Georg and Lappe, 2007). If such perceptual distortions could be demonstrated in an animal model, the activity of single neurons could be monitored while these illusions occur. We believe that FEF is a promising brain region in which to study these phenomena. FEF is relatively feature-independent (Mohler et al., 1973) and connected to subcortical structures such as the basal ganglia and cerebellum (Alexander et al., 1986; Stanton et al., 1988b; Lynch et al., 1994)—areas thought to play critical roles in timing (Ivry and

Spencer, 2004; Matell and Meck, 2004; Jahanshahi et al., 2006). Finally, FEF is a critical component of the oculomotor circuit needed to maintain transsaccadic spatial stability (Sommer and Wurtz, 2002, 2006). It may be that this same pathway assists in maintaining temporal stability before and after eye movements (Yarrow et al., 2004b).

3.0 MAGNITUDE ENCODING OF TIME INTERVALS IN PREFRONTAL CORTEX

3.1 ABSTRACT

It is frequently assumed that the timing of an event is faithfully represented by the timing of responses in sensory neurons. In many animals and systems, this is undoubtedly true. But this may be an incomplete description of how the primate visual system represents the timing of visual events. In primates, analysis of the visual scene occurs at short timescales because periods of fixation are frequently interrupted by eye movements. Fixation periods last for roughly half a second which, as discussed in the previous chapter, is also the time range during which neuronal adaptation (time-dependent changes in the size of successive responses) takes place. The primate visual system, therefore, does not need to rely solely on response latencies to determine temporal intervals between stimuli. Visual response magnitudes carry useful information as well. Our hypothesis was that the primate brain makes use of this magnitude information.

To test the magnitude hypothesis, we trained monkeys on a temporal discrimination task and recorded from neurons in the prefrontal cortex and midbrain. Monkeys performed well on the task but, as expected, made errors when the intervals to be discriminated were similar in duration. We found that their errors in time judgment were accompanied by changes in visual response magnitude in prefrontal cortex that matched the coding inherent in neuronal adaptation. Monkeys incorrectly judged short intervals as “long” when the second, adapted visual response

was larger than normal, and they incorrectly judged long intervals as “short” when the second response was smaller than normal. We found few, if any, changes in prefrontal or midbrain response latencies that could account for the monkeys’ errors. These findings support the magnitude hypothesis but not the latency hypothesis for sub-second time intervals. Thus, the magnitude of adapted prefrontal activity seems to act as an additional source of timing information about events in the world.

3.2 INTRODUCTION

It is frequently assumed that the timing of an event is faithfully represented by the timing of responses in the visual system. This assumption is based on laboratory studies in which a visual stimulus is presented and sensory neurons respond by firing at a relatively fixed latency (Alpern, 1954; Ikeda and Wright, 1975; Schmolesky et al., 1998). Such studies are usually designed to isolate neuronal responses in time from one another. During natural behavior however, this is rarely the case. Primates move their eyes roughly three times per second (Henderson and Hollingworth, 1998; DiCarlo and Maunsell, 2000; Berg et al., 2009). These actions interrupt the incoming stream of visual information (Matin et al., 1972; Campbell and Wurtz, 1978). Thus, the visual system naturally parses the world into sequential stimuli at intervals of hundreds of milliseconds.

Sub-second visual stimulation has two dramatic effects on visual activity. First, given the overall shorter amount of time, visual responses are more likely to overlap one another. This temporally-compressed visual activity makes it difficult to determine where one response ends and the next one begins. Such ambiguity may be a problem for a system that relies on response

latencies for information about sub-second timing of events in the world. Second, as stimuli are presented closer together in time, the magnitudes of successive responses decrease. This effect, known as neuronal adaptation, is ubiquitous throughout the visual system (Chapter 2, Judge et al., 1980b; Brown et al., 1987; Robinson and Kertzman, 1995; Lisberger and Movshon, 1999; Motter, 2006; Lehky and Sereno, 2007; Mayo and Sommer, 2008).

Despite these constraints at the neuronal level, the visual system must accurately interpret successive visual responses in order to track the timing of events in the world. The most parsimonious possibility is that neurons represent the timing of events solely by the timing of associated responses (“latency hypothesis”), despite the increase in variability caused by compression and adaptation at naturalistic timescales. An alternative possibility is that the brain exploits the timing information implicit in adaptation to help disambiguate time intervals. In this scenario, the size of the second visual response (and subsequent responses) would be used as a measure of the immediately preceding interval (“magnitude hypothesis”).

We tested the magnitude and latency hypotheses by training monkeys to report the amount of time between two consecutive flashes of light, presented at intervals that approximate those experienced during natural behavior. We recorded the activity of single neurons in prefrontal cortex, an area of the brain implicated in both conscious visual, and time, perception (Kim and Shadlen, 1999; Thompson and Schall, 1999; Thompson and Schall, 2000; Onoe et al., 2001; O'Shea and Walsh, 2004; de Lafuente and Romo, 2005; Genovesio et al., 2006; Li and Lisberger, 2011; Libedinsky and Livingstone, 2011). We also recorded from neurons in the midbrain for comparison. We found that, on average, time judgments were accompanied by changes in visual response magnitude consistent with those seen in neuronal adaptation; larger visual responses were associated with “long” judgments and smaller responses with “short”

judgments. This magnitude effect was found in prefrontal neurons, but was weaker or absent in midbrain neurons. These findings indicate that the timing of events in the world at brief, naturalistic timescales may be at least partially encoded by the magnitude of corresponding neuronal activity.

3.3 METHODS

3.3.1 Subjects and neuronal recordings

Two adult male monkeys (*Maccaca mulatta*) were surgically prepared for neuronal recordings in the frontal eye field and superior colliculus, as previously described (Chapter 2). All procedures and experiments were conducted under the supervision of the University of Pittsburgh Institutional Animal Care and Use Committee and complied with the guidelines set forth in the United States Public Health Service Guide for the Care and Use of Laboratory Animals.

Data collection procedures were identical to those detailed in the previous chapter (Chapter 2; Mayo and Sommer, 2008). Briefly, FEF and SC were identified based on a combination of stereotaxic location, structural MRI, microstimulation, and established physiological properties. The superficial layers of SC were defined as the initial 1 mm of transient visual responses upon first entering the SC. For acceptance into our dataset, neurons in the intermediate layers of SC had to be at least 1 mm below the top of the SC (average 1-3 mm). Intermediate SC neurons were generally recorded only after encountering other neurons that showed saccade-related activity. We purposefully did not explore the deeper SC but we cannot rule out that some neurons from the deep layers were included in our “intermediate SC” sample.

Receptive field (RF) centers were determined via a memory-guided or visually-guided saccade task using stimuli iteratively presented at eight target directions (cardinal and diagonal directions) and at least eight amplitudes (1-35°). In all areas, neurons were selected for recording only if they were visually responsive in the RF mapping task.

Although intSC and supSC are functionally distinct regions of SC, they are layers of the same brain structure. For the sake of discussion only, we refer to them as different brain areas below.

3.3.2 Visual stimuli and behavioral task

Monkeys performed a two-alternative temporal discrimination task (Fig. 11). To start each trial, the monkey fixated a spot (0.3° square, 6 cd/m²) in the center of the screen (fixation window: 1.5-2.2°). After ~750 ms, the fixation point was turned off and a saccade cue (0.3° square) appeared 12° to the right. After the saccade, the monkey maintained fixation (1.8-2.5° window) for 500-1300 ms before two identical stimuli (50 ms duration each, 294 cd/m²) were flashed consecutively in the RF center. We manipulated the amount of time between the two stimuli (stimulus onset asynchrony; SOA) on each trial. We used intervals comprised of two flashes (as opposed to the duration of a single flash) to facilitate the measurement of changes in neuronal activity, where response onsets are more robust than offsets. SOAs ranged from 250-450 ms at ~16.7 ms intervals. Stimulus size was scaled approximately with cortical magnification at increasing RF eccentricities (“M-scaling”; Rovamo and Virsu, 1979; Dow et al., 1981). After the two stimuli were flashed, a second delay period (200-1000 ms) occurred. The extended, randomized delay periods surrounding stimulus presentation were used to dissociate the time

interval from the timing of other events in the task. After the delay, two choice targets appeared in the visual field ipsilateral to the recording hemisphere; one target 6° above, the other 6° below the initial fixation point (now extinguished). Monkeys indicated whether the time interval's SOA was shorter or longer than a learned reference interval of 350 ms by making a saccade to one of the two choice targets. For monkey K, 'short' choices were indicated by a saccade to the upper green choice target and 'long' choices were indicated by saccades to the lower red choice target. The response mapping was reversed for monkey C. Choice targets were photometrically isoluminant (40 cd/m^2 ; Photo Research Inc., Model PR-655). The saccade target location was fixed within a block of trials. If the rightward saccade target was close to the RF center, the saccade target and relative choice target locations were rotated 45° away from the RF, so that the initial saccade was either up-right or down-right. Thus, the initial saccade was always at least 45° degrees away from the RF. Correct judgments were reinforced with a liquid reward, and incorrect judgments and aborted trials resulted in a brief timeout and no reward.

When the reference interval of 350 ms was presented, there was no correct answer. On these trials, reward was randomly dispensed at a 50% rate. On trials with low-contrast stimuli (50% full contrast; 146 cd/m^2), which were randomly interleaved during the time interval discrimination task, reward rate approximated the animal's mean performance on all trials up to that point. We used this reward method because an arbitrary 50% reward rate was not sufficiently motivating during training. Low contrast trials comprised less than 20% of the total number of trials, and activity during those trials is excluded from all analyses in this document.

During a given recording session, we presented five or six time intervals ("test intervals" for that session) to each neuron. Selected test intervals were chosen on a daily basis to obtain a range of behavioral performance. The set of test intervals during a session was always

symmetrical around the reference interval. For example, whenever an SOA of 300 ms was used (a “short” trial 50 ms less than the reference interval), the complementary “long” SOA of 400 ms (50 ms greater than the reference interval) was also always used. When the reference interval was presented, only five intervals were used: two “short” intervals, two “long” intervals, and the reference interval. There were small (< 2 ms), highly significant differences in reaction time for each monkey as function of trial success (rank sum test, monkey K: correct RT $>$ incorrect RT, $p < 0.001$; monkey C: incorrect RT $>$ correct RT, $p < 0.001$).

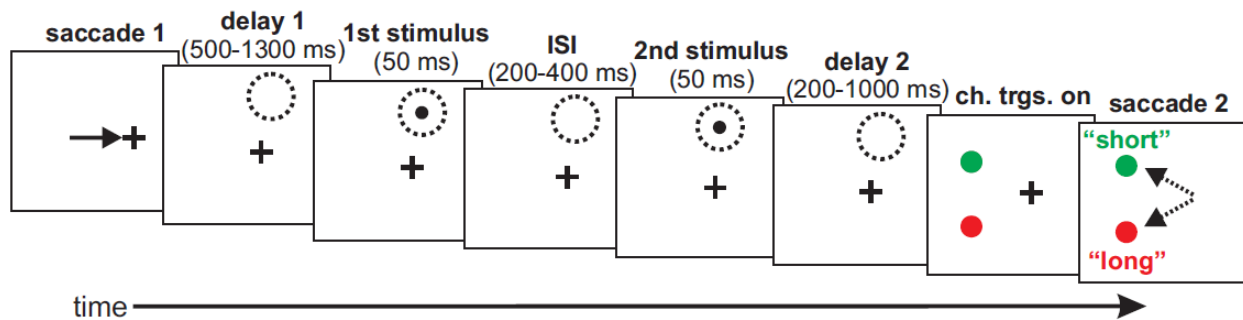


Figure 11. Time interval discrimination task

Task began with fixation in the center of the screen (not shown). The interstimulus interval (ISI) refers to the amount of time between the offset of the first visual stimulus and the onset of the second visual stimulus. Dashed circle represents the neuron’s RF (not visible to monkey). Arrows represent saccade vectors. “ch. trgs. on” indicates time of choice targets’ onset. Stimulus onset asynchrony (SOA) = ISI + 50.

3.3.3 Data analysis

Neuronal activity and response epochs

Visual activity was measured during standard visual epochs, 50-150, 30-130, and 20-70 ms after stimulus onset, for FEF, intSC, and supSC respectively. In all areas, saccade-related activity was measured -50 to 50 ms around saccade onset and delay activity was measured 100 ms before

second stimulus onset, during the interstimulus interval. Baseline activity was measured 100 ms before first stimulus onset. All response epochs were verified using averaged population responses. A minimum of four trials was required for inclusion in a particular condition's dataset. Increasing this minimum to 10 trials did not alter our main results but resulted in less statistical power at extreme time intervals where fewer incorrect trials occurred. We found no major differences in recording locations or between animals and therefore combined data for neuronal analyses. We used 10 ms-wide Gaussians to create all spike density functions (MacPherson and Aldridge, 1979). We also used 5 ms-wide Gaussians for latency analyses (see below).

We collected an average of 268 completed trials per neuron (\pm 7 standard error of the mean; SEM), yielding 28,100 total trials from monkey K and 22,618 total trials from monkey C. Correlations, coefficients of determination (r^2), and associated p-values were calculated using the `corrcoef` function in MATLAB (Mathworks). Nonparametric statistical tests were used whenever we could not ensure that the data were normally distributed.

Population responses

Before combining for population measures, individual neurons were normalized to their peak visual response to the first visual stimulus across all trials. Difference signals were created by subtracting incorrect from correct population responses at each time interval and averaging the resulting activity. We tested for a significant difference between “short” and “long” difference signals using a permutation test. At each analysis window time point (window width 100 ms, step size 20 ms) starting at first stimulus onset, we measured the difference in firing rate between the means of the two difference signal populations. We then took the original firing rates of the

two groups and randomly reassigned the choice labels (“short” and “long”). We measured the difference between the two new permuted population means, and repeated this process of reassignment and subtraction 1000 times. The original firing rates were considered significantly different in a particular analysis window if the original difference in means fell outside the 95% interval of the permuted difference in means. A similar procedure was used to test for differences between distributions of choice probabilities (see below).

Choice probability

Following signal detection theory (Green and Swets, 1988), we used receiver operating characteristic (ROC) methods to measure the discriminability of distributions of firing rates by an ideal observer (Parker and Newsome, 1998). Firing rates were sampled in an epoch 1000 ms long following first stimulus onset. For each neuron, we normalized activity for each presented time interval by removing its baseline activity and dividing by its standard deviation (“z-correction”). We arranged all ROC statistics such that if the Area Under the ROC curve (AUC) was greater than 0.5, then “long” choice trials contained higher firing rates. We calculated the AUC for each time interval for each neuron. Choice probability (CP) was then defined as the AUC. Grand choice probability (Britten et al., 1996; Nienborg and Cumming, 2006) was calculated by determining the AUC for the distributions of firing rates across all trials for a given neuron, sorted according to “short” or “long” choices. Grand choice probability (CP) was then defined as the AUC across time intervals for each neuron.

Statistical significance of CP distributions, differences between them, and grand CP values per neuron were evaluated using permutation tests. For example, differences between the means of two CP distributions (e.g., FEF vs. intSC) were calculated using a permutation test in

which CP values from the original distributions were randomly assigned to each group (“FEF” or “intSC”) while maintaining the original sample sizes. After each re-assignment, a new difference in mean CP distribution values was calculated. This process was repeated 1000 times. The original difference was deemed significant if it fell outside of the 95% interval of the permuted “null distribution” (n=1000) of mean CP differences. A similar test was used to determine if a given neuron’s grand CP was significantly different from 0.5, except that the firing rates from all trials were randomly assigned to the original number of “short” and “long” choices and AUC values recalculated. The original grand CP was deemed significant if 0.5 fell outside of the 95% interval of the null distribution of AUC values.

Latency

We calculated visual response latencies by separately using spike density functions (SDFs) and raw spike counts. Our main two methods relied on analysis of the SDFs. We compiled the trials from each condition (time interval x correct/incorrect) and created the neuron’s SDF for that condition’s activity. The first latency measure, “SD2”, defined visual response onset as the point in time, relative to stimulus onset, in which the SDF rose two standard deviations above the baseline activity level. Brief (< 5 ms) activity above baseline was ignored. An analogous measurement was used to measure response *offset* except that we measured the time after peak firing rate in which the activity declined to two standard deviations above the baseline firing rate, relative to stimulus offset. The second latency measure, “Peak”, defined visual response onset at the time required for the response to reach its maximum firing rate during the visual epoch, relative to stimulus onset. SD2 and Peak latency values were calculated for each of the two visual responses in every condition, relative to stimulus onset. An additional measure of

response latency, “MaxDiff” defined the response latency as the time at which the visual response and baseline activity maximally differed (Sripati and Olson, 2009). It involved sliding an analysis window, of variable sizes up to the size of the visual epoch, over the entire visual response. Activity in this response window was iteratively compared (via t-test) to an equivalent window during the baseline activity. Results from this spike-based latency measure were consistent with our SDF latency measures (Table 2). Correlations between methods were strong and highly significant in all areas (weakest correlation was in intSC between MaxDiff and Peak, $r^2 = 0.73$, $p < 0.0001$). We therefore relied exclusively on the SD2 and Peak methods because they were computationally less demanding.

There was the potential to find a latency effect because of related changes in magnitude. (However, we did not find such a latency effect; see Results.) Our Peak metric was therefore particularly useful because it was not prone to finding latencies based on changes in the strength of visual responses (a so-called “iceberg effect”), unlike other latency measures based on statistical criteria. Accordingly, a correlation between the latency and magnitude of first visual responses in FEF reached significant levels using the SD2 method (using SDF = 10 ms; p -value of correlation coefficient = 0.04), but was not significant for the Peak method (using SDF = 10 ms; $p = 0.70$). To be conservative, we considered both magnitude and latency changes separately.

We also used a bootstrap method on the peak latency values of both first and second visual responses for all neurons. We used the Peak metric for bootstrapping because it provided an unambiguous time point for all neurons in the dataset. For each condition (correct or incorrect trials for each time interval) for each neuron, we randomly sampled with replacement the original number trials. We calculated the latency of the visual response for each resampled

set of spike times. We repeated this procedure 1000 times to obtain a distribution of latency values expected by chance given the original spike times. The standard deviation of this null distribution was then used as a measure of the standard error of the original latency value (Efron and Tibshirani, 1998; Herrington and Assad, 2010).

Control tasks

When isolation allowed, we used two additional tasks to investigate the basic visual and saccadic activity contained in each neuron. The first was a memory-guided saccade task, detailed in Chapter 2. Visual and saccadic responses in the memory-guided saccade task were used to calculate a neuron's visuomotor index (VMI; see Results). The second task was a delayed visually-guided saccade task. Stimulus locations in this task were altered on a daily basis to match the locations used during that day's time interval discrimination task. This second task was used to test for saccade-related responses into the ipsilateral visual hemifield. It mirrored the second half of the time interval discrimination task, but omitted the presentation of the time interval. Thus, monkeys were simply required to fixate and saccade to a target when it was illuminated and after the fixation point was extinguished. The fixation point was at the same location as the fixation point in the discrimination task that day (where the monkey fixated when stimuli were flashed in RF). The saccade targets were at the same locations as those of the choice targets in the discrimination task. Saccade target locations were randomized between trials, and monkeys were rewarded for saccading to the illuminated target.

Microsaccades

Microsaccades were defined as small, saccade-like deviations in eye position during fixation.

Following previous work (Rolfs et al., 2008; Hafed et al., 2009), microsaccades had to meet the following criteria: eye position velocity greater than 8 deg/sec, surpass an acceleration threshold of 650 deg/sec², and last for longer than 8 ms (Engbert and Kliegl, 2003). Because we were primarily interested in changes in neuronal activity close to when the time intervals were presented, we only measured microsaccades during the second fixation period, after the initial saccade offset until just before the saccade to one of the choice targets.

3.4 RESULTS

3.4.1 Monkeys are capable of fine temporal discrimination

Monkeys were trained to perform a time interval discrimination task (Fig. 11). On each trial, we varied the amount of time between two sequential flashes of light (stimulus onset asynchrony; SOAs: 250-450 ms at 16.7 ms intervals) presented during fixation. This range of times was chosen for two reasons: to approximate timescales regularly encountered by the visual system during natural behavior, and to elicit adapted but still easily detectable neuronal responses (Chapter 2; Mayo and Sommer, 2008). The monkey was rewarded for making a saccade to one of two choice targets, “short” or “long”, based on that trial’s SOA relative to a learned reference interval of 350 ms. The first saccade was used to control for motor activity that would otherwise be effectively random due to initial target acquisition during free viewing in the inter-trial interval. Errors in performance increased as the presented time intervals approached the duration of the reference interval. This provided us with a sufficient number of error trials, critical for carrying out our analyses.

Behavioral performance during each recording session was quantified in terms of percentage of trials in which the monkey selected the “long” choice target at each time interval. Behavioral data across all sessions were fit with a cumulative Gaussian function (e.g., Nienborg and Cumming, 2006; Cohen and Maunsell, 2010) for each monkey (Fig. 12). Although SOAs incremented by only ~17 ms and varied within a total range of 200 ms, monkeys’ performance scaled with task difficulty (i.e., proximity of SOA to reference interval), indicating that they could readily discriminate differences in time. These results extend previous findings of broader time discrimination in monkeys (Leon and Shadlen, 2003; Janssen and Shadlen, 2005; Chiba et al., 2008; Lebedev et al., 2008; Genovesio et al., 2009; Zarco et al., 2009), and show that monkeys can also perform fine temporal discrimination at short, naturalistic timescales.

3.4.2 Predicted changes in neuronal activity as a function of behavioral performance

While monkeys performed a time interval discrimination task, we recorded from 90 neurons in the frontal eye field, 47 neurons in intermediate layers of the superior colliculus, and 9 single neurons and 43 multiunit groups in superficial layers of the superior colliculus (respectively, monkey K: 41, 31, 9/21; monkey C: 49, 16, 22). Because single-unit and multiunit recordings from superficial SC displayed similar patterns of activity, the data were pooled ($n = 52$).

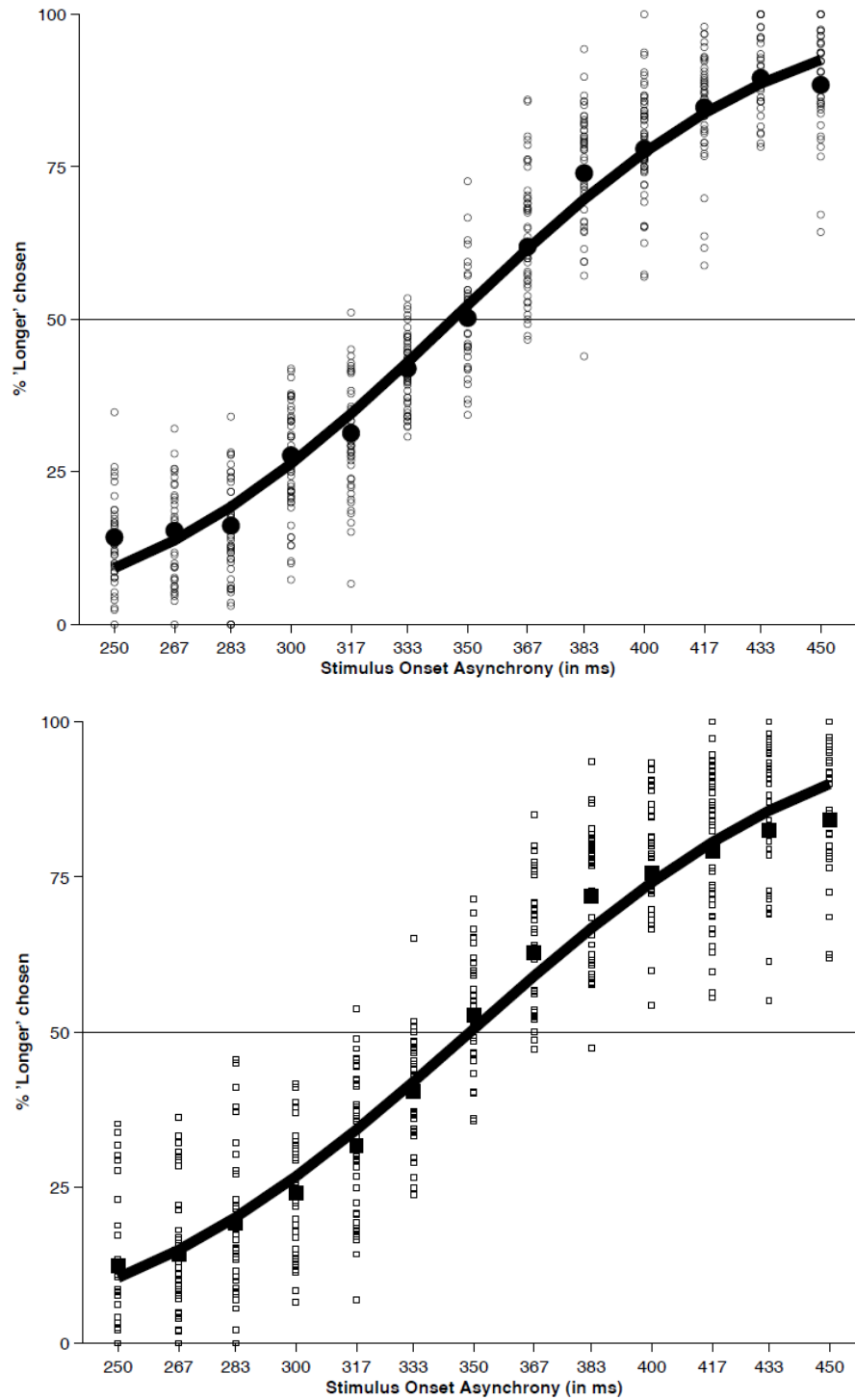


Figure 12. Behavioral performance for each monkey

Percentage of “long” choices as a function of presented time interval for monkey K (top) and monkey C (bottom) over all recording sessions. Small symbols indicate performance for time intervals in individual sessions. Psychometric curve is fit to the average performance (large circles) across SOAs. Symbols (circles for monkey K and squares for monkey C) are maintained whenever possible for the remainder of the chapter.

In light of previous work that correlated activity in FEF to conscious visual perception (Kim and Shadlen, 1999; Thompson and Schall, 2000; Libedinsky and Livingstone, 2011), we were interested in analyzing the differences in FEF responses between correct and incorrect trials. The latency hypothesis predicts that the *time* between visual responses—resulting from the two stimuli that define the time interval—will differ according to the monkey’s behavioral report. For example, consider the case where the monkey is presented with a “short” interval (i.e., $SOA < 350$ ms) but it incorrectly chooses the “long” choice target. According to the latency hypothesis, the error would be due to an increased amount of time between visual responses (“response onset asynchrony”; ROA) on that trial, reflecting the “long” choice. Overall, the latency hypothesis predicts that incorrectly judged “short” trials will have longer ROAs than their correct counterparts, and incorrectly judged “long” trials will have shorter ROAs than their correct counterparts (Fig. 13, bottom row). From the outset, we remain agnostic as to whether the latency hypothesis applies to the onset, peak, or offset of visual responses.

In contrast, the magnitude hypothesis predicts that the *strength* of visual activity should differ between correct and incorrect trials. We were specifically interested in testing the idea that the size of visual responses would represent time intervals according to the code implicit in neuronal adaptation. Smaller visual responses would be associated with shorter time intervals and larger responses with longer intervals. Within the context of our task, if the magnitude hypothesis is true then we would expect to see larger second visual responses in incorrect “short” trials when compared to correct “short” trials, and smaller second visual responses in incorrect “long” trials when compared to correct “long” trials (Fig. 13, top row). Thus, like the latency hypothesis, the “magnitude hypothesis” predicts specific changes related to correct versus incorrect performance that should be complementary in “short” and “long” trials.

Finally, note that the magnitude and latency hypotheses are not mutually exclusive. They refer to different properties of the neuronal response (strength or latency). It is therefore possible that one or both properties could change independently of each other on a given trial. Our idea of a magnitude hypothesis is specifically guided by the code inherent in neuronal adaptation. But other magnitude codes are also possible. For example, incorrect trials across all time intervals could be caused by diminished spatial attention. In this were the case, we would expect to see concomitant decreases in the size of activity across all intervals on incorrect trials (Thiele et al., 1999; Cook and Maunsell, 2002), even if attention is dynamically allocated over time (Ghose and Maunsell, 2002). The actual magnitude code could even be opposite to that implied by neuronal adaptation (i.e. smaller responses for longer perceived durations), perhaps as an effort by the neuronal system to counter adaptation. However, we reserve the term “magnitude hypothesis” specifically for magnitude changes in the direction seen in neuronal adaptation. Finding evidence of any other magnitude code would refute our adaptation-based version of the magnitude hypothesis.

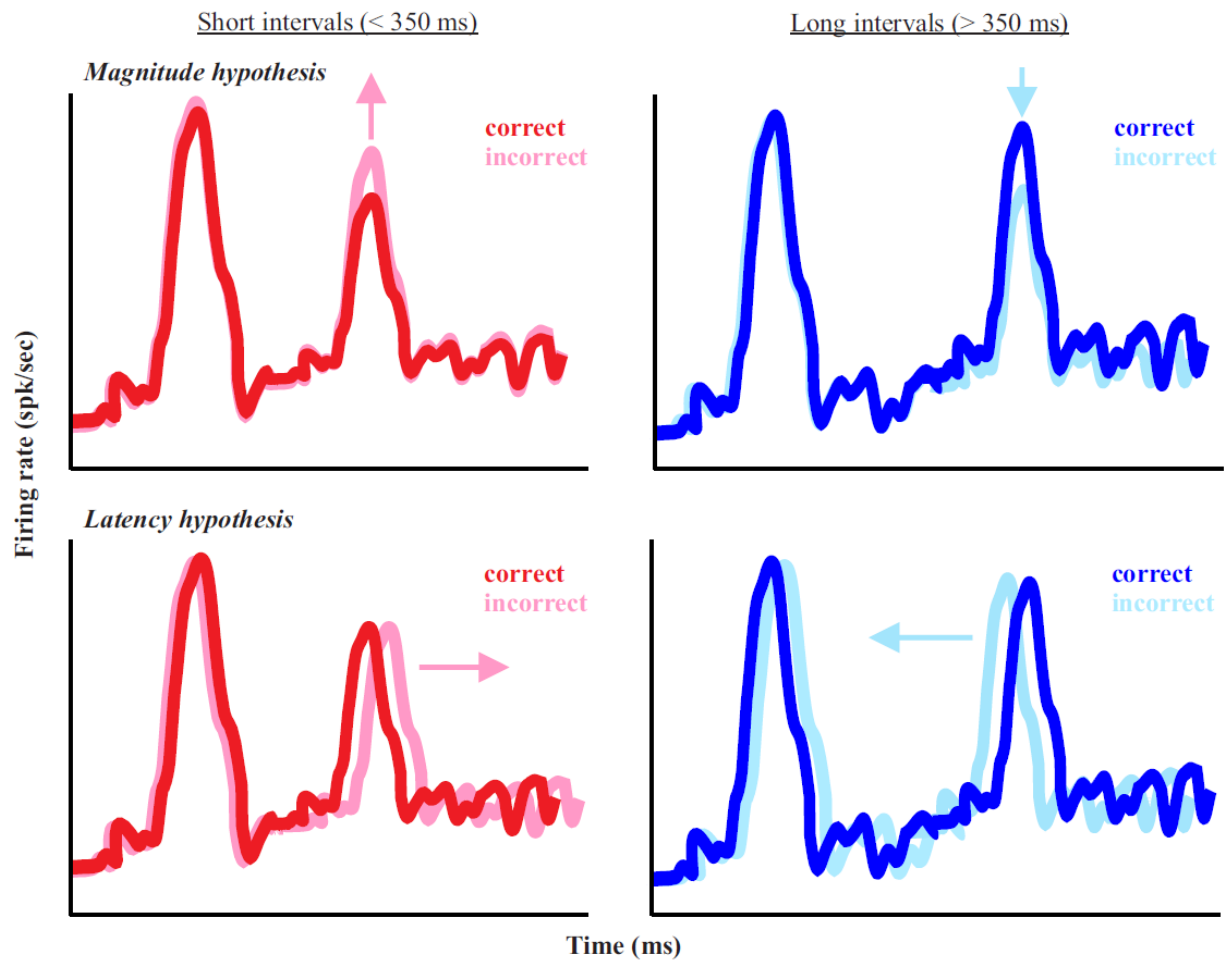


Figure 13. Predicted changes in neuronal activity by the magnitude and latency hypotheses

The magnitude (top row) and latency (bottom row) hypotheses make contrasting predictions for time intervals relative to the reference interval (350 ms). Predicted responses are shown for “short” (left column; red) and “long” (right column; blue) population activity. Activity during incorrect trials is indicated by lighter colors. Arrows indicate the direction of change predicted by each hypothesis for each time interval group.

3.4.3 Changes in response magnitude predict time discrimination performance in prefrontal cortex

We first asked whether changes in the size of visual responses correlated with the animals' abilities to discriminate brief time intervals. Figure 14 shows correct (dark traces) and incorrect (light traces) responses of an example FEF neuron to four different time intervals; two short SOAs (red) and two long SOAs (blue), relative to the reference interval of 350 ms. This neuron showed larger second visual responses in “short” incorrect trials, and a smaller response for one of the two “long” incorrect trials (the other was unchanged). This neuron's responses to the first visual stimulus were roughly equivalent, although not identical. Thus, the changes in visual responses followed the direction of changes seen in neuronal adaptation (e.g., larger responses are perceived as longer) as predicted by the magnitude hypothesis.

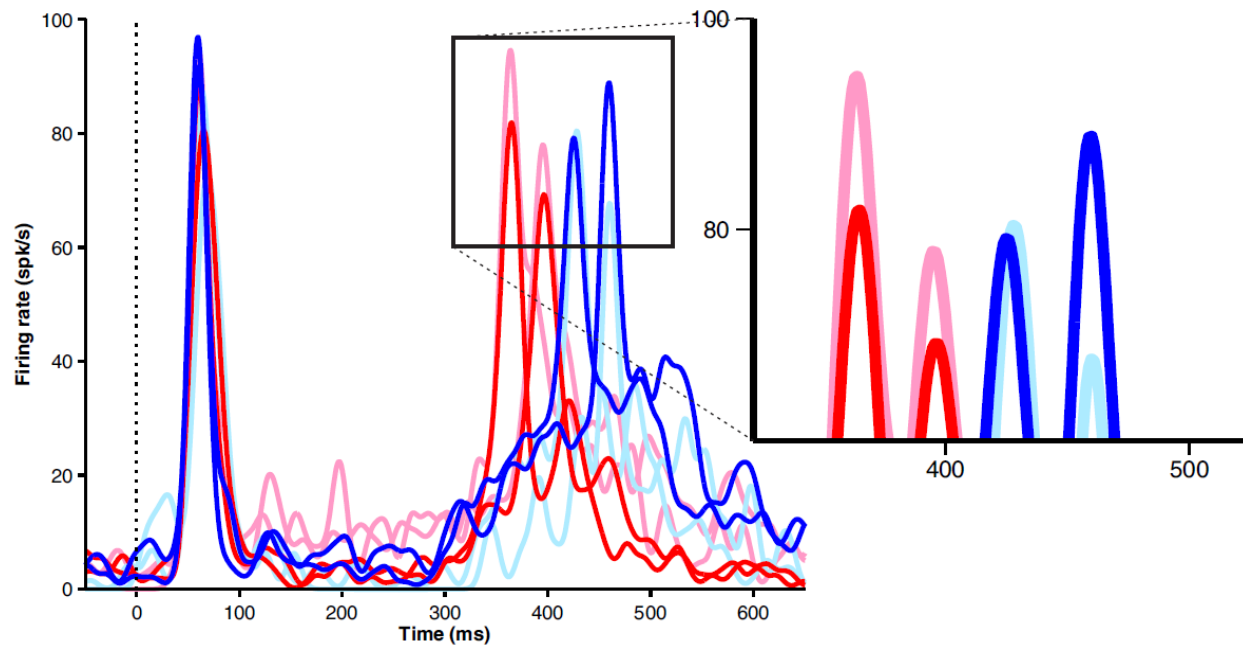


Figure 14. Responses of an FEF neuron to four time intervals

Neuronal responses to “short” intervals (red) and “long” intervals (blue). SOAs = 300, 333, 367, and 400 ms. Lighter colors indicate activity during incorrect trials. Responses to the reference interval are omitted because they cannot be classified as “correct” and “incorrect”. Activity is aligned to first stimulus onset at time zero (dashed vertical line).

To directly measure changes in FEF second visual responses on a neuron-by-neuron basis, we grouped visual responses at individual time intervals based on their relationship to the reference interval of 350 ms. Thus, intervals less than 350 ms (250-333 ms) were classified as “short” and intervals greater than 350 ms (367-450 ms) were classified as “long”. We only included neurons and time intervals that had a sufficient number of both correct and incorrect trials (four or more trials per interval; see Methods). We counted the average number of spikes during the visual response epoch for every time interval for each neuron. We then compared spike counts for correct versus incorrect trials as a function of interval group (“short” and “long”).

The same pattern of magnitude changes seen in the example neurons was also significant in the population of FEF neurons (Fig. 15). On average, second visual responses for short time intervals were significantly *larger* in incorrect trials than in correct trials (Fig. 15A). For long time intervals, second visual responses were significantly *smaller* in incorrect trials than in correct trials (Fig. 15B).

One assumption of the magnitude hypothesis is that first responses are largely equivalent, while changes in the second visual response vary with adaptation and may correlate with time perception. In support of this idea, the systematic changes found in FEF second visual responses were not seen in first visual responses (Fig 15C, D). There were no significant differences in first response firing rates between correct and incorrect trials, for either the “short” or “long” groups. A comparison of the analyses of first versus second visual response suggests that FEF neurons modulate their activity more during the second visual response. This result is consistent with the magnitude hypothesis, where timing information is implicitly embedded in size of the second response while the first response remains largely unchanged (see Chapter 2).

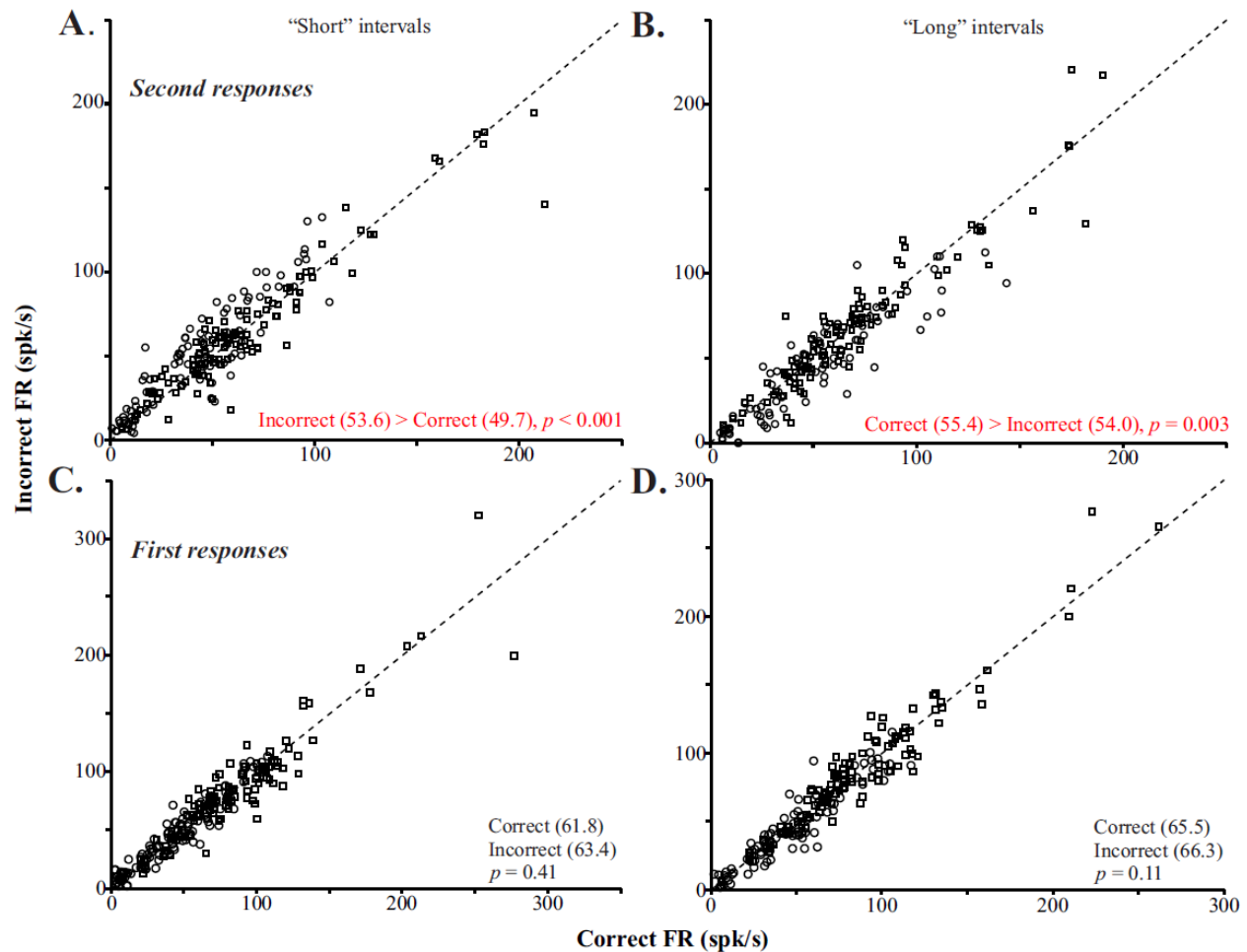


Figure 15. Correct versus incorrect spike counts during visual responses in FEF

A, B FEF responses to **second** visual stimulus. C, D FEF responses to **first** visual stimulus. Responses are grouped relative to the reference interval (“short” trials in left column, “long” trials in right column). Median firing rates (spikes per second) shown in parentheses. Each symbol represents responses at a single time interval for a given neuron; a single neuron can therefore have up to 2-3 points per plot. Dashed diagonal lines are unity lines. P-values are for paired Wilcoxon signed rank test. As in Figure 12 above, data from monkey K = circle, monkey C = square.

Changes in second response magnitude as a function of behavioral performance (correct or incorrect) were found not only in the pooled data but at individual SOAs as well (Fig. 16). For all six “short” time intervals, the mean second visual responses were larger in incorrect than in correct trials (Fig. 16, red). We found the opposite effect for “long” time intervals (Fig. 16, blue). In five out of six “long” SOAs, the mean second visual responses were larger in correct

than in incorrect trials. The sixth SOA showed no change. These differences were significant in three individual time intervals (Fig. 16, stars; rank sum test, $p < 0.05$), and approached significance in two other intervals (SOA 333: $p = 0.058$; SOA 383: $p = 0.051$). Pooled “short” and “long” differences were significantly different from zero (correct minus incorrect, 1-sample signrank test, short: $n = 215$, median = -2.92, $p < 0.001$; long: $n = 195$, median = 2.12, $p = 0.003$). The same analyses performed on first visual responses yielded no significant differences at any time intervals (rank sum test, $p > 0.05$) or pooled differences (1-sample signrank tests, $p > 0.05$).

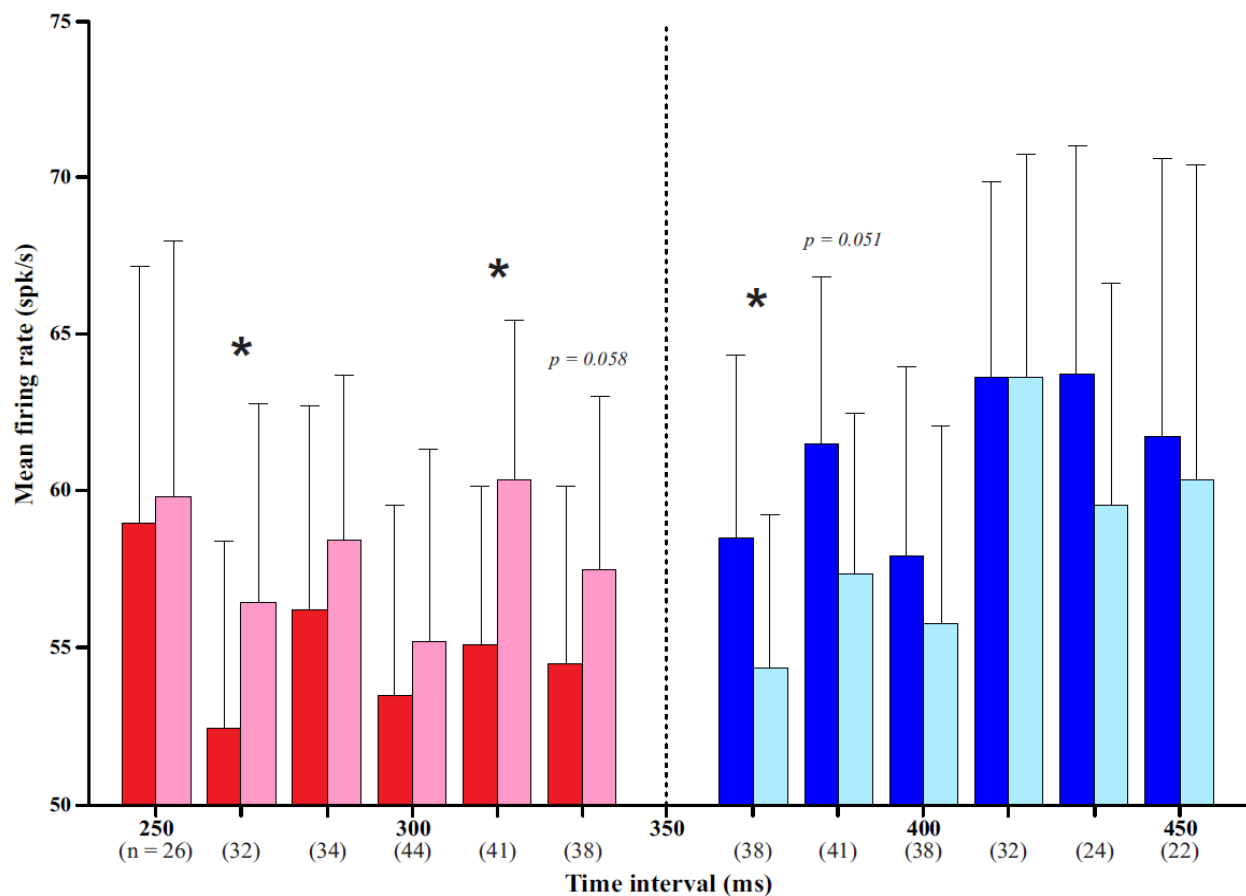


Figure 16. Mean firing rate across all FEF neurons at each time interval, for short and long intervals

Lighter shades indicate activity from incorrect trials. Sample sizes are listed below each interval in parentheses. Stars indicate a significant difference between correct and incorrect firing rates (Wilcoxon signed rank test, $p < 0.05$).

Finding significant differences at this stage of analysis was notable for two reasons. First, individual neurons varied greatly in their baseline firing rates and visual responsiveness, leading to large variability when activity was averaged together. Second, only a subset of time intervals were tested for each neuron (see Methods and Fig. 16) since neuronal isolation can be maintained for only ~1 hour typically. This restriction meant that each SOA had relatively modest sample sizes, which limited the statistical power of tests performed on an interval-by-interval basis. We found no significant changes in second responses as a function of task performance across time intervals within each group (Kruskal-Wallis test, short: $p > 0.2$; long: $p > 0.4$). These straightforward analyses suggest that responses in both short and long time intervals show changes in response strength predicted by the magnitude hypothesis.

To assess whether our findings were specific to FEF or more widespread in the visual and saccadic systems, we also recorded from the superior colliculus (SC). This multi-layered structure effectively allowed us to sample two brain areas (the superficial and intermediate layers) in a single electrode penetration. FEF and SC neurons show a similar range of visual-motor activity, and the two areas are anatomically linked via a single synapse in the thalamus (Lynch et al., 1994; Sommer and Wurtz, 2004), among other pathways.

We repeated the population analyses for our 47 intSC neurons and 52 supSC combined neurons and multiunit groups. We found no support for the magnitude hypothesis in intSC neurons. Correct and incorrect second visual responses were not significantly different for either short or long time intervals (Table 1, row 1, right columns). The same analyses on first visual responses were also not significant (Table 1, row 1, left columns). In supSC neurons, we found contradictory evidence. Second visual responses in “short” SOA trials reached significance (Wilcoxon signed rank test, $p = 0.03$) in correct versus incorrect trials, in the direction opposite

from that predicted by the magnitude hypothesis (Table 1, row 2, 3rd column). First visual responses in “long” SOA trials also reached significant ($p = 0.02$; Table 1, row 2, 2nd column).

Table 1. Differences in the magnitude of visual responses, correct versus incorrect, in intSC and supSC.

Median firing rates (spikes per second) are shown in parentheses. P-values are for paired Wilcoxon signed rank test. Significant p-values are bolded.

	First responses		Second responses	
	<u>Short</u>	<u>Long</u>	<u>Short</u>	<u>Long</u>
intSC	Correct (44.2) Incorrect (42.9) $p = 0.64$	Correct (43.8) Incorrect (46.7) $p = 0.49$	Correct (36.7) Incorrect (35.0) $p = 0.53$	Correct (38.1) Incorrect (38.5) $p = 0.35$
supSC	Correct (52.3) Incorrect (55.6) $p = 0.18$	Correct (57.1) Incorrect (52.0) $p = 0.02$	Correct (39.3) Incorrect (33.8) $p = 0.03$	Correct (48.0) Incorrect (43.1) $p = 0.68$

We plotted population spike density functions for “short” and “long” time intervals to verify our analyses on spike counts and to examine changes in activity over time. FEF population activity showed clear changes in the size of second visual responses depending on whether the presented time interval was shorter or longer than the reference interval (Fig. 17, top). Population responses also revealed that the magnitude changes related to the monkeys’ success on each trial emerged during the second visual response and were sustained after the stimuli turned off. To highlight the relative difference in FEF activity between correct and incorrect trials, we subtracted incorrect activity from correct activity and created “difference

signals” for the short and long intervals (Fig. 17, bottom). The magnitude hypothesis predicts that the “short” difference signal should be positive (correct activity > incorrect activity) and the “long” difference signal should be negative (incorrect activity > correct activity).

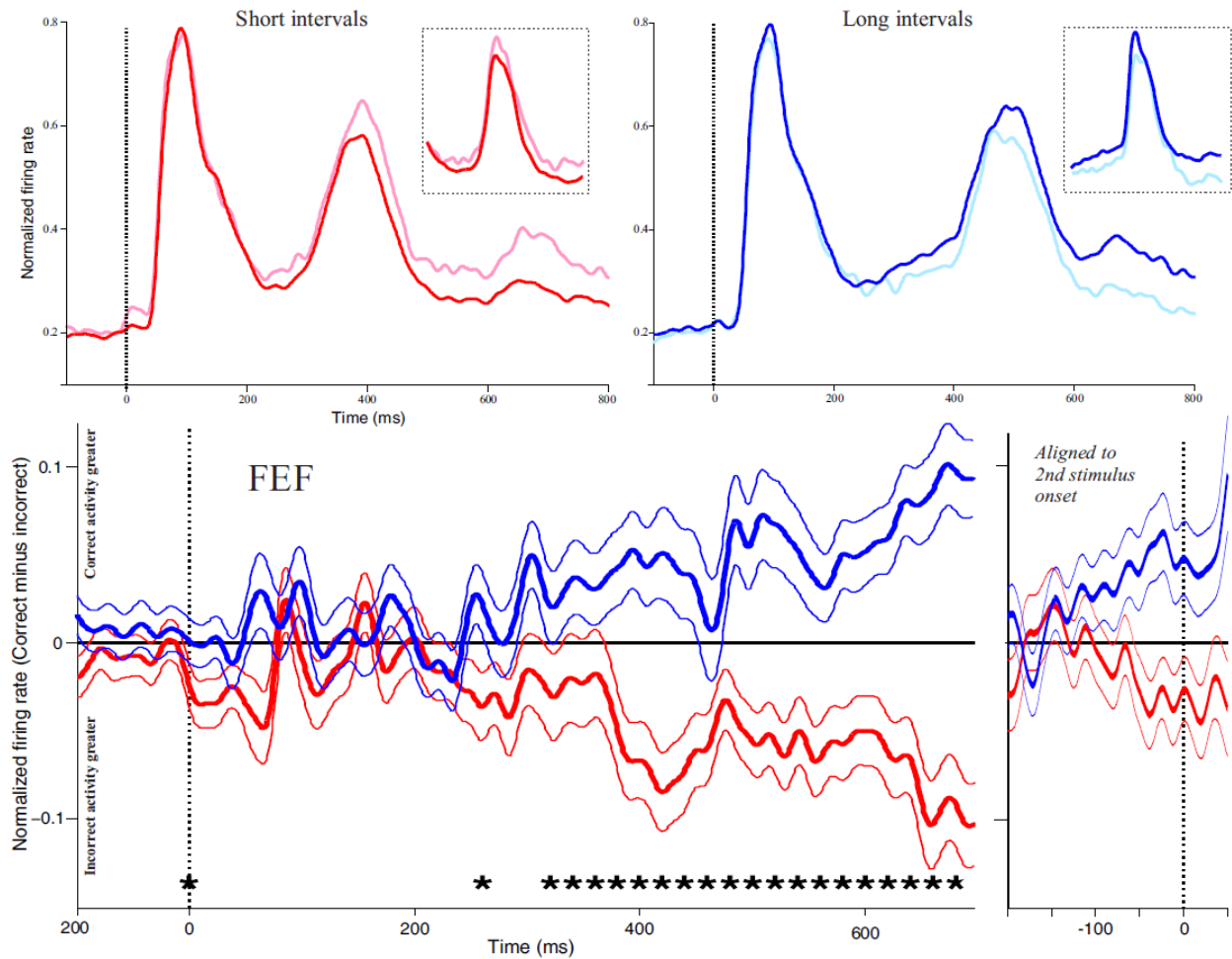


Figure 17. Population responses in FEF

Top, Responses aligned to **first** stimulus onset (dashed vertical line). *Top left*, “short” time intervals (SOAs < 350 ms) in red. *Top right*, “long” time intervals (SOAs > 350 ms) in blue. Lighter colors indicate activity on incorrect trials. Insets show second visual responses aligned to **second** stimulus onset. *Bottom left*, differences in correct and incorrect population activity short and long intervals aligned to **first** stimulus onset (dashed vertical line at time 0). Thin lines represent \pm standard error of the mean. Stars indicate differences between short and long difference signals (100-ms wide bins, slid every 20 ms). *Bottom right*, same activity aligned to **second** stimulus onset (dashed vertical line at time 0).

Difference signals (Fig. 17, bottom) in FEF showed a clear separation in the “short” versus “long” responses around the time of the second visual stimulus. This separation was maintained after the second stimulus was turned off. In contrast, intSC and supSC difference signals showed transient separations around the time of visual responses (Fig. 18). These small fluctuations were likely artifacts of the low baseline firing rates (i.e., lower noise) in SC, which led to apparent differences if the dynamics of responses (i.e., rise time and decay of visual responses) did not exactly match.

One striking aspect of the difference signals in FEF is the time at which activity begins to separate. The standard errors of both “short” and “long” difference signals diverge from zero around 350 ms after first stimulus onset, the time equal to the duration of the reference interval. One possible interpretation of this results is that “short” and “long” categories are established at this time, in agreement with recent work showing that FEF neurons are capable of representing a given task’s decision boundary (Ferrera et al., 2009). However, we are cautious about making such a claim at this point. First, the temporal resolution of the analysis is likely not precise enough to distinguish signal changes on the order of ~17 ms, the interval increment used in the task. Second, because monkeys naturally performed better at intervals further away from the reference interval (Fig. 12), fewer neurons contributed to the population analysis at extreme SOAs. Weighting population activity with neuronal responses closer to the reference interval would therefore effectively bias any estimates of changes in the time course of activity. We return to this issue of the time course of discriminatory signals below (Results: choice probability).

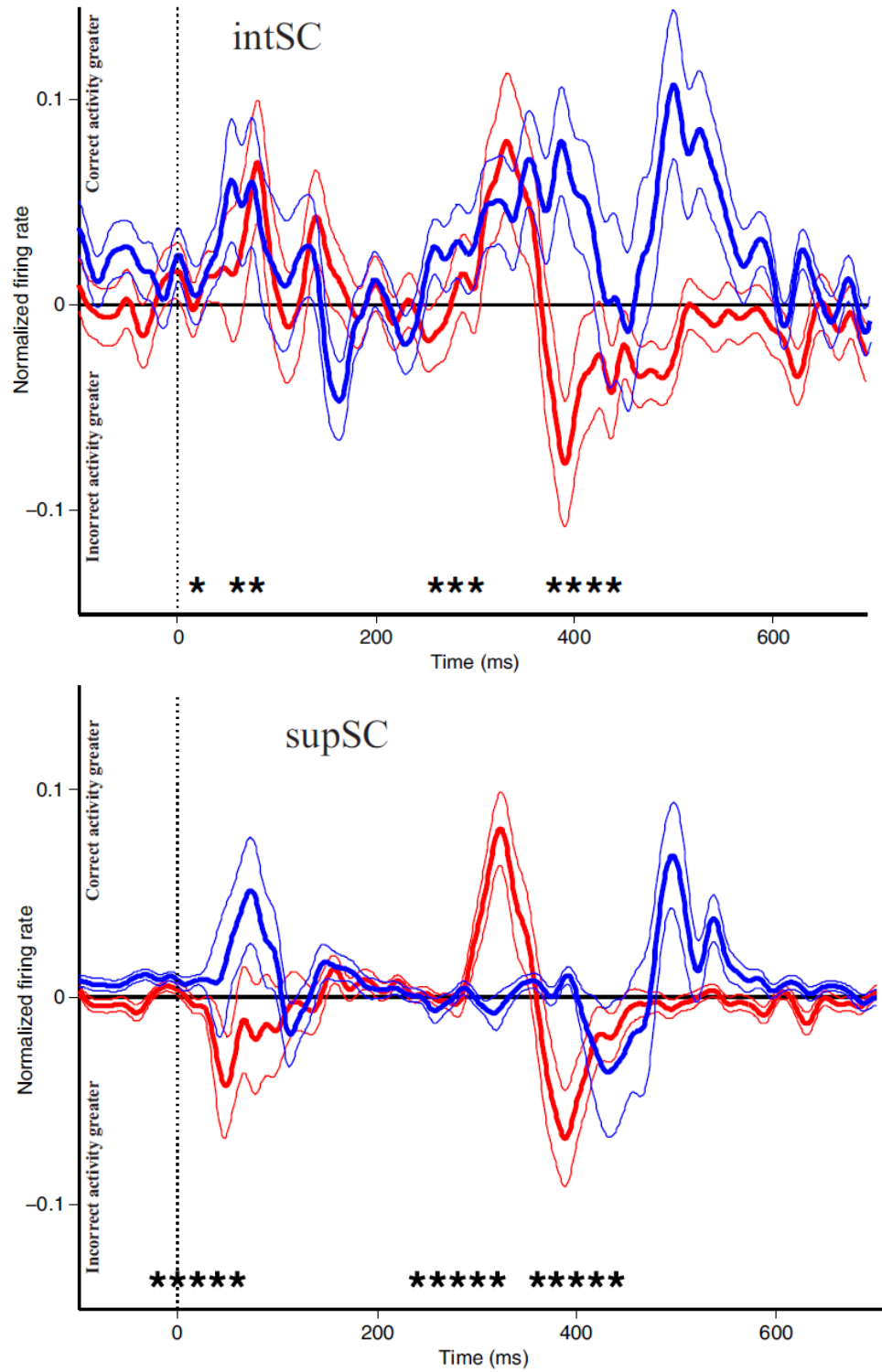


Figure 18. Population responses in SC

Correct minus incorrect population activity for short (red) and long (blue) time intervals in intSC (top) and supSC (bottom), aligned to first stimulus onset. Same conventions as Figure 17, bottom.

Whereas changes in “short” and “long” signals could be determined by looking at deviations in each SEM from zero (i.e., no difference), we could look more quantitatively at changes in activity of short and long interval population responses relative to each other. We ran a permutation test on the grouped difference signals at 20 ms increments (in 100-ms long windows). At each time point, we tested whether the “short” and “long” signals significantly differed from one another (black stars in Figures 17 and 18). Our permutation results confirm the timing and duration of activity discussed above; FEF activity first diverged and remained separated at ~330 ms, while SC activity briefly fluctuated in both positive and negative directions during visual responses before returning to zero. Thus, all population plots illustrated clear changes in the size of FEF’s second visual responses based on the monkey’s ability to discriminate the presented time intervals. These FEF results are entirely consistent with the changes predicted by the magnitude hypothesis, while we saw little evidence for the magnitude hypothesis in intSC and supSC neurons.

We also note that the sustained activity between first and second responses (or “delay activity”) is modulated much like the visual responses are in Figure 17 (bottom). This point is discussed in more detail in the last section of the Results.

3.4.4 Choice probability

In addition to analyzing averaged population data, we also wanted to test whether magnitude changes in FEF neurons could encode time interval perception on a trial-by-trial basis. We used ROC analysis to determine the likelihood that an ideal observer could predict the monkeys’ performance given the trial-by-trial firing rates of a particular neuron. We focused on

the first 1000 ms of neuronal activity following first stimulus onset to include both visual responses and early delay activity on all trials. (Late visual responses in the longest time interval could appear at ~600 ms.) For each neuron, we normalized activity at each time interval by subtracting the neuron's average baseline activity and dividing by its standard deviation. This correction minimized population bias effects caused by individual neurons with exceptionally high firing rates or variable responses. Critically, we organized the analysis such that choice probability (CP) values greater than 0.5 corresponded to firing rate changes consistent with the magnitude hypothesis regardless of time interval (see Methods for details).

We first calculated the CP for individual time intervals for each neuron. This process yielded 441 FEF interval-neuron combinations (Fig. 19). (Five or six intervals were presented to each neuron, and we excluded incorrect conditions with fewer than four trials as well as reference interval presentations where, by definition, trials could not be classified as correct or incorrect.) The mean of the CP distribution in FEF was significantly greater than 0.5 (permutation test, mean = 0.56, $p < 0.001$). Thus, FEF single-trial firing rates could be used to predict behavioral choices at a level significantly better than chance and in accordance with the magnitude hypothesis. The means of CP distributions in SC (Fig. 20) were both lower than that of FEF and only one was statistically significant (Fig. 20; intSC: mean = 0.52, $p = 0.04$; supSC: mean = 0.51, $p = 0.06$).

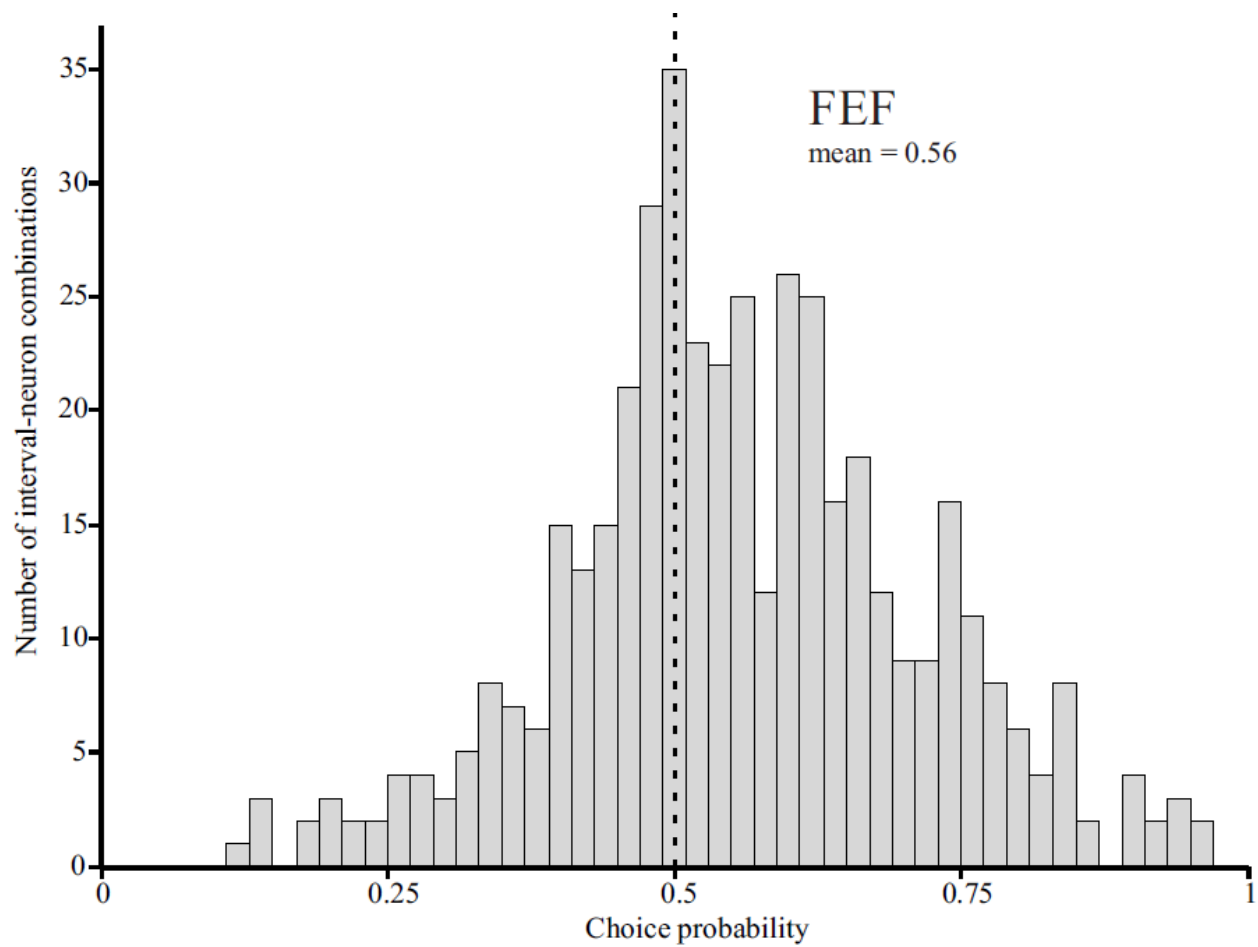


Figure 19. Distribution of choice probabilities for all FEF interval-neuron pairings

Values greater than 0.5 indicate changes consistent with the magnitude hypothesis. $n = 441$ from 90 neurons.

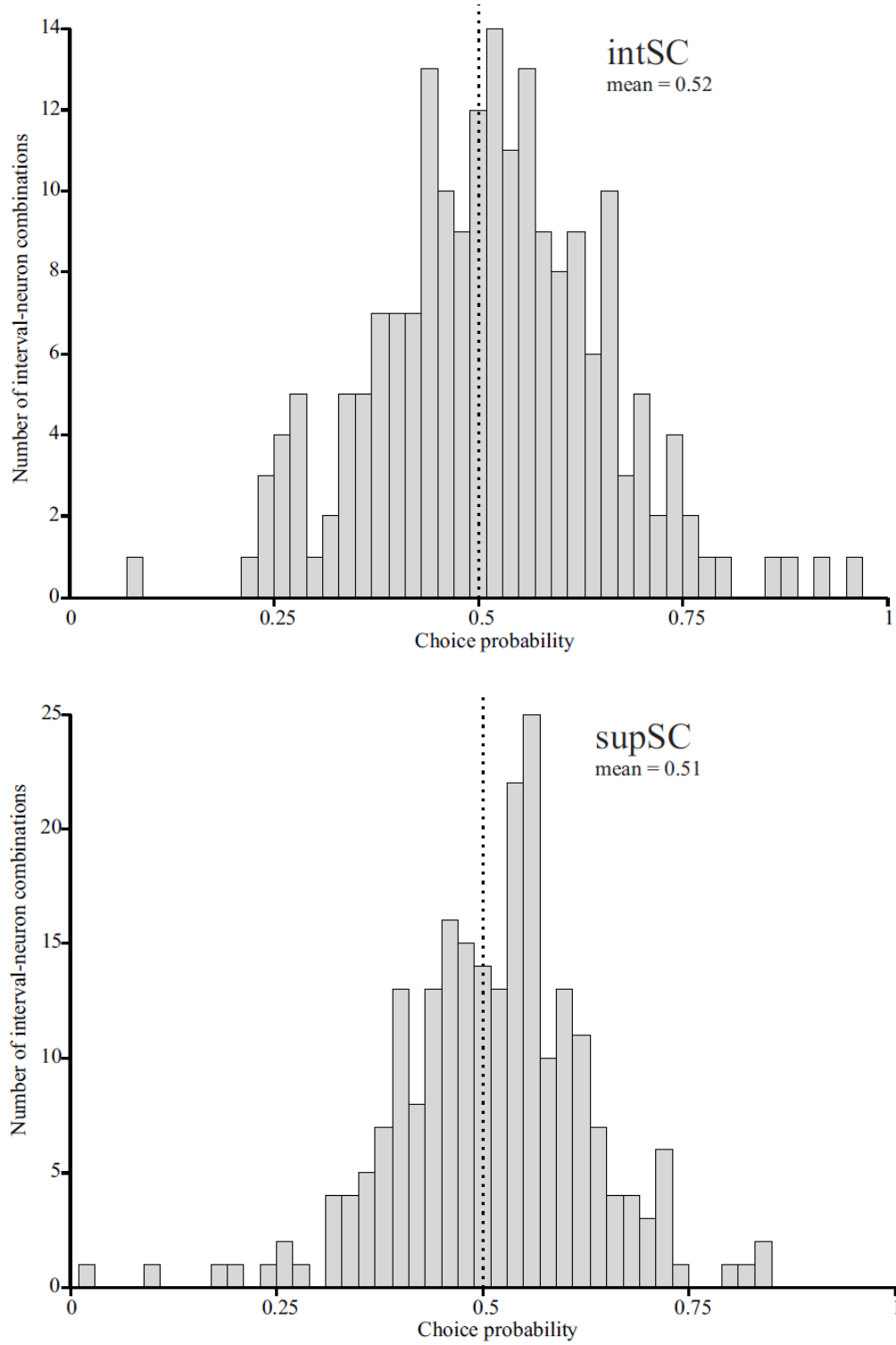


Figure 20. Distribution of choice probabilities for all SC interval-neuron pairings

CP values in intSC (top): $n = 194$ from 47 neurons. CP values in supSC (bottom): $n = 230$ from 52 neurons and multiunit groups.

We then asked if the CPs varied according to the presented time interval. Mean CPs in FEF were above 0.5 for all time intervals (Fig. 21, blue triangles) and significantly greater than 0.5 in 8 out of 12 SOAs (1-sample t-test; $p < 0.05$, circled triangles in Fig. 21). These mean CPs did not significantly vary across time intervals (one-way ANOVA, $p = 0.8$), indicating that the ability to discriminate time intervals based on changes in firing rate (greater FR for incorrect “short” and correct “long” trials) was robust across time intervals. In SC, the CPs hovered around 0.5 and showed large, but not significant deviations further away from the reference interval (Fig. 21, intSC: crosses, supSC: x’s). Like FEF, intSC and supSC showed no effect of time interval on mean CP (one-way ANOVA, intSC: $p > 0.06$; supSC: $p = 0.41$).

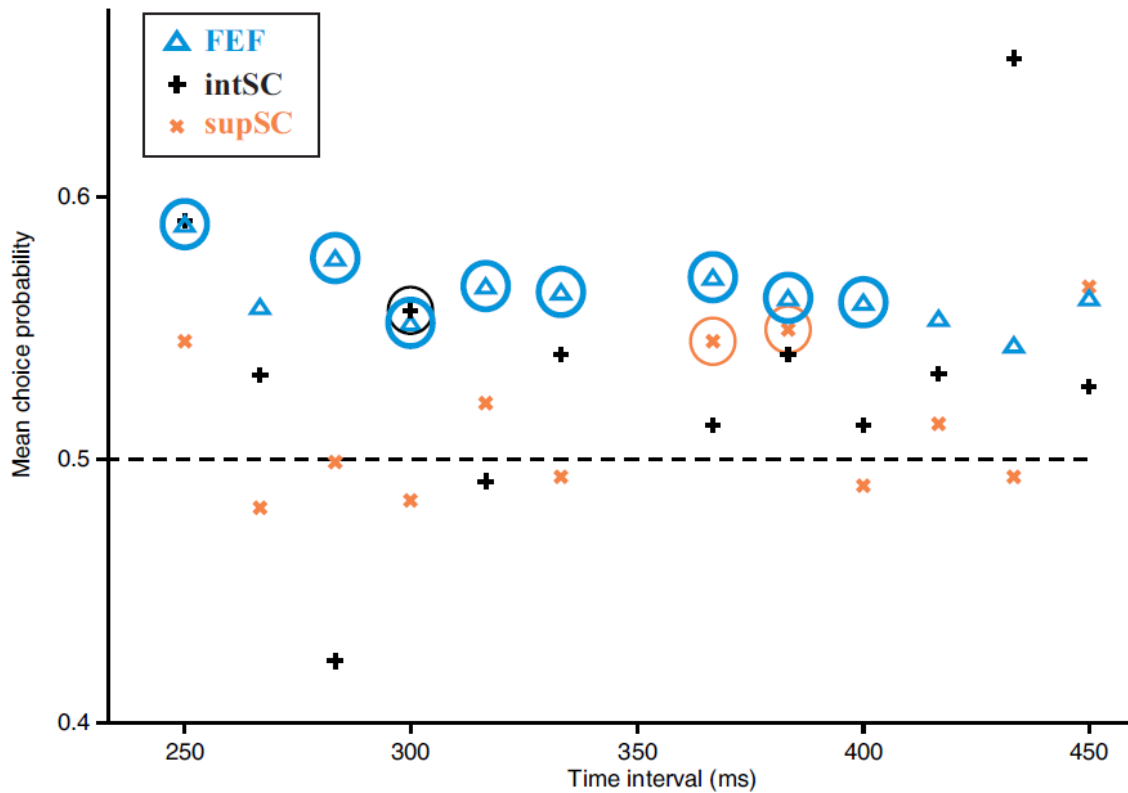


Figure 21. Population grand choice probability as a function of time interval

All FEF choice probability values were above 0.5, consistent with the magnitude hypothesis. SC choice probabilities were scattered. CPs significantly different from 0.5 are circled.

Because FEF and SC neurons showed no significant change in choice probability across stimulus intervals, we pooled the responses for each neuron and recalculated the CP. To gain a more complete picture of each neuron's discriminative abilities, we included trials in which the reference interval was presented, after sorting those trials according to choice ("short" or "long"). This process yielded a single "grand choice probability" (Britten et al., 1996) for each neuron. (For reference, the representative FEF neuron in Figure 14 had a grand CP of 0.67.)

FEF (Fig. 22) contained a significantly greater number of neurons with significant grand CPs than either intSC (Fig. 23, top) or supSC (Fig. 23, bottom). Forty-two percent (38/90) of FEF neurons had grand CPs significantly different from 0.5, in contrast to 11 % (5/47) in intSC (Fig. 23, top) and 4% (2/52) in supSC (permutation test, $p < 0.05$). The mean grand CP across all FEF neurons (0.54) was significantly greater than 0.5 (permutation test, $p < 0.001$). The FEF distribution was also significantly greater than both the intSC and supSC distributions (permutation test, intSC: $p < 0.01$; supSC: $p < 0.001$). Grand CP populations in SC did not significantly differ from one another (intSC mean = 0.51, supSC mean = 0.50; permutation test, $p > 0.05$) or from 0.5 (permutation test, intSC: $p = 0.13$; supSC: $p = 0.21$). In sum, when compared to both intSC and supSC, FEF had a greater number of individually significant neurons and, across the populations, FEF showed a significantly greater capacity to represent the monkeys' perceptual choices across time intervals. Therefore, among the three areas tested in our time interval discrimination task, meaningful choice predictive activity was unique to FEF.

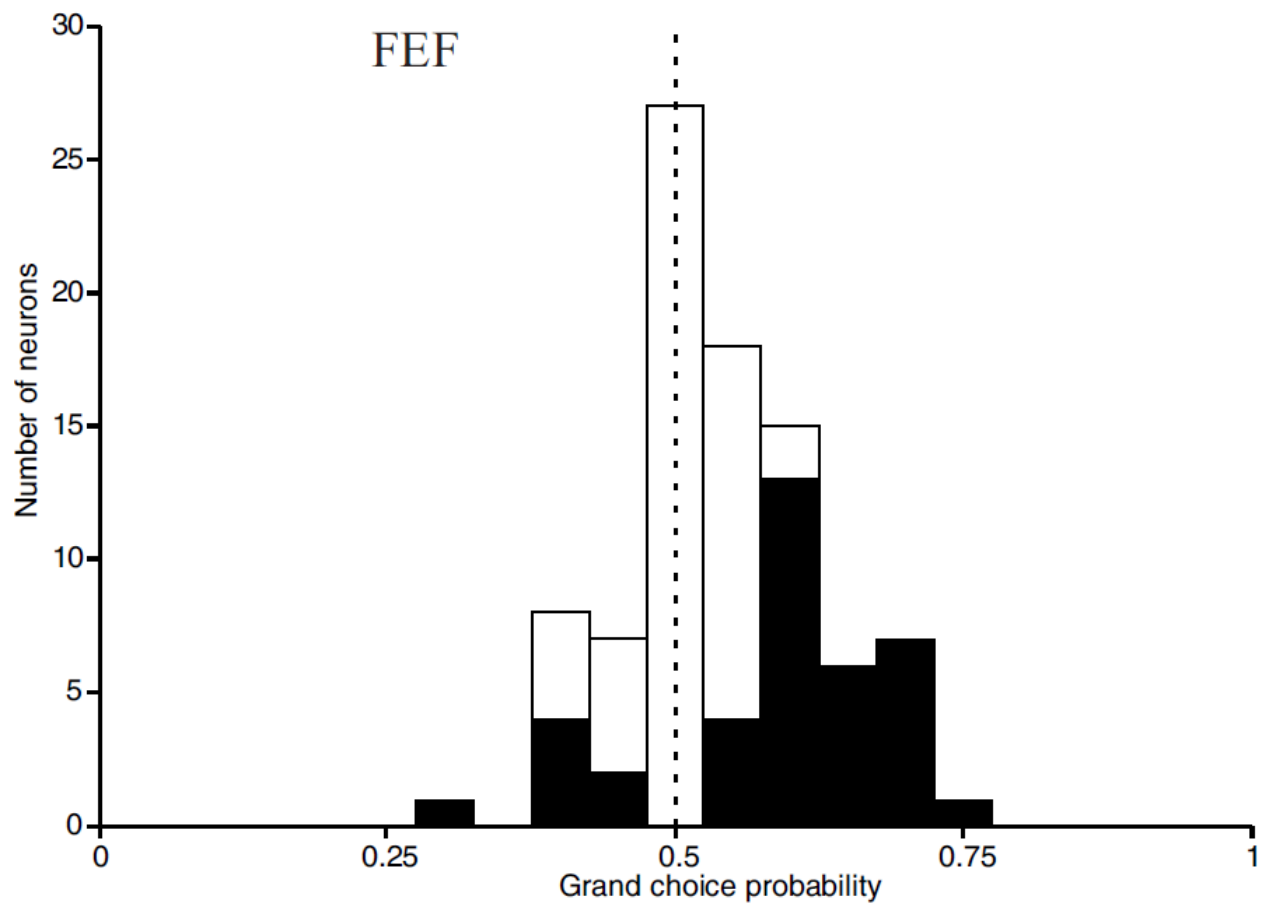


Figure 22. Grand choice probability distributions in FEF.

Black bars indicate neurons that are significantly different from 0.5 ($n = 38$; 25 from monkey K, 13 from monkey C).

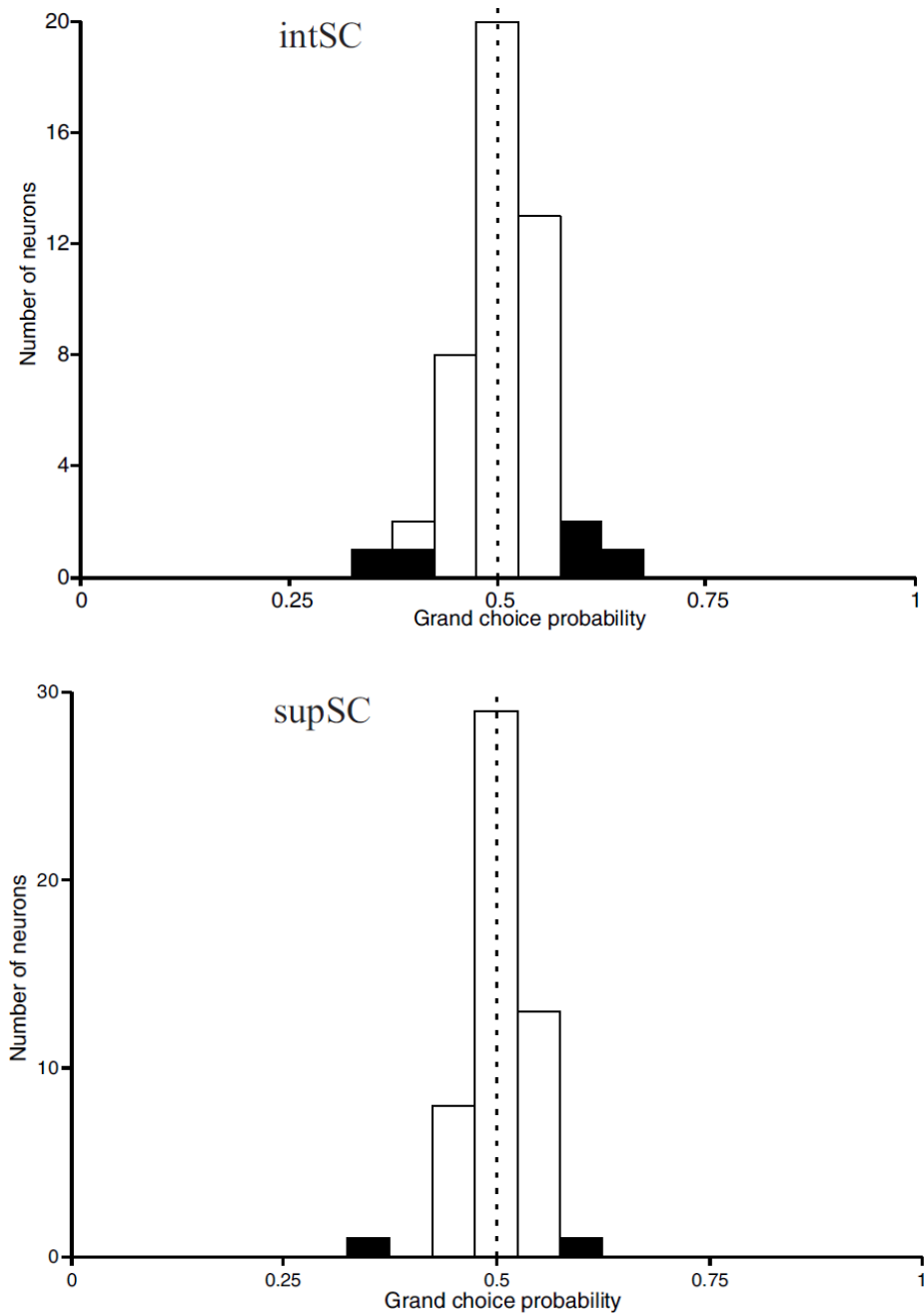


Figure 23. Grand choice probability distributions in SC.

Black bars indicate neurons that are significantly different from 0.5 (intSC: $n = 5$; 3 from monkey K, 2 from monkey C; supSC: $n = 2$, 1 from each monkey).

Our time interval discrimination task was fairly unique in that we only presented “anchor” points for sensory discrimination. Our sequential stimuli demarcated the start and end of the value to be discriminated (the temporal interval) but there was no experimental stimulation in the intervening time. Thus, unlike most decision tasks in which sensory evidence is accumulated during the stimulus duration (e.g., Britten et al., 1996; Cohen and Newsome, 2008), evidence accumulation in our task was not directly stimulus driven (i.e., the screen was blank except for the fixation point). Instead, for trials to be completed correctly, evidence accumulation during this period was likely driven by an internal representation of the passage of time during the interstimulus interval. As a result, we would then expect the CP—as a measure of the decision-making process—to also increase over time. To determine if this was the case, we again used grand CP as a measure of individual neurons’ abilities to discriminate across time intervals, according to changes in firing rate predicted by the magnitude hypothesis. We calculated the grand CP on a cumulative basis starting at first stimulus onset. We plotted the population’s average CP over increasingly longer time periods, increasing by 50 ms intervals (Fig. 24).

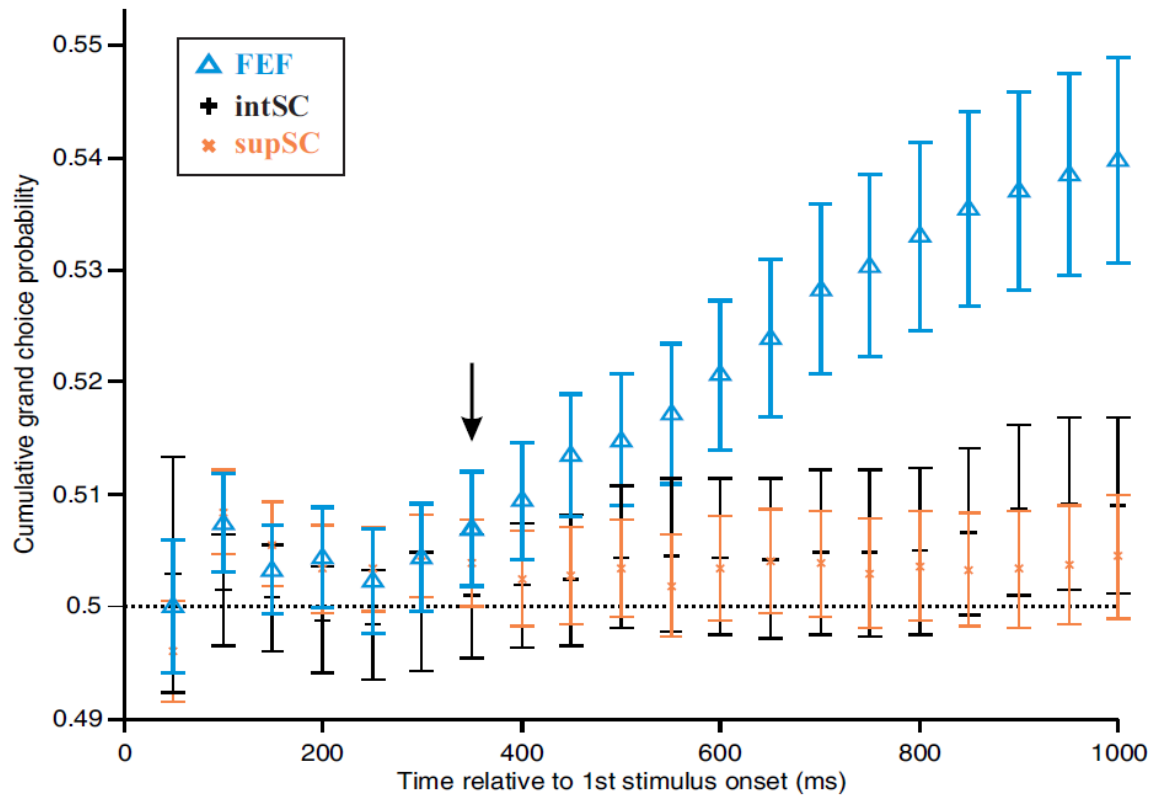


Figure 24. Cumulative choice probability reveals that FEF choice-related activity accumulates over time

Cumulative CP values for each area at increasingly longer intervals (50 ms intervals). Each symbol represents the population's CP calculated from time zero up to that point in time in the task. Black arrow indicates first time of sustained significance in FEF. Error bars indicate \pm SEM.

FEF neurons first showed significant choice-related activity at 350 ms. This result corroborates the timing of FEF difference signals shown earlier (Fig. 17, bottom). Importantly, these FEF results were not simply the result of accumulating any neuronal activity over time. If that were true, we would have expected to see similar changes in intSC and supSC, as well as a steady increase in the CP of FEF immediately after first stimulus onset. Instead, cumulative CP in FEF remained relatively flat until it reached ~ 350 ms, at which point activity became, and remained, significantly different from 0.5. Along the same lines, the time of significant choice-related activity in FEF was unlikely simply the result of motor preparation because we would

have expected to see a similar change in intSC, given its comparable visuomotor properties (Mohler and Wurtz, 1977; Sommer and Wurtz, 2004). (We address concerns about motor activity in detail below).

Population and choice probability results both suggested that FEF neurons encoded information about the behavioral outcome of each trial, but SC neurons did not. We were encouraged by the similar time courses of activity described by the differential population responses (Fig. 17, bottom) and cumulative CP data (Fig. 24) for FEF. Both analyses showed relatively undistinguishable activity during the first visual response, followed by a significant inflection (i.e., a potential “choice”) around ~350 ms after first stimulus onset. These neuronal dynamics followed the demands of the task, which required time accumulation to start immediately after first stimulus onset and task success depended on keeping track of the time between visual stimuli. Because we used a range of time intervals centered on the reference interval of 350 ms, we saw significant choice-related activity near this time point in the averaged population data. Furthermore, differences in correct and incorrect activity emerged sooner in the “long” trials than in the “short” trials (Fig. 17, bottom right). This was likely because, in “long” trials only, after 350 ms had passed the monkey knew it was a “long” trial, regardless of the exact time of the second visual stimulus. The onset of differential activity in “long” trials therefore occurred sooner relative to that of “short” trials when aligned to second stimulus onset, in accordance with the task design and the monkeys’ behavior.

3.4.5 Changes in response latency are an unlikely explanation for behavioral performance

We showed that in FEF neurons larger visual responses were correlated with behavioral reports of longer time intervals and smaller visual responses were correlated with reports of shorter time intervals. This neuronal modulation occurred after the first visual response around the time of the second visual response and was maintained after the visual stimuli turned off. But the analyses on response magnitude ignored a potentially simpler mechanism for representing time, namely, modulating the timing of visual responses (“latency hypothesis”; Fig. 13, bottom row).

Our main goal in testing the latency hypothesis was to calculate the time between the two visual responses resulting from the two stimuli that defined each trial’s time interval. Our analyses were based on the prediction—implicit in the latency hypothesis—that longer intervals between visual responses should correlate with behavioral reports of longer time intervals, and shorter intervals between responses should correlate with reports of shorter time intervals. That is, there should be less time between visual responses in correct compared with incorrect “short” trials, and more time between visual responses in correct compared with incorrect “long” trials. We carried out a range of analyses on visual response latencies. To summarize, for all methods in all areas we found no support for the latency hypothesis. Before concentrating on the amount of time between elicited neuronal responses, we first review findings regarding the latencies of single visual responses in all three areas.

General properties of visual response latencies

We relied primarily on two measures of visual response latency: the time required to reach two standard deviations above baseline firing rate (“SD2”) and the time to reach peak firing rate (“Peak”; see Methods). We used the SD2 method to detect both response onsets (as above) and

offsets. Response offsets were defined as the time when response dropped to two standard deviations above baseline after peaking. Like in the section above regarding the magnitude hypothesis, we fit spiking data with a 10-ms wide Gaussian kernel. In this section only, we also computed latency measures on data convolved with a 5-ms wide Gaussian to help ensure that negative results were not simply due to smoothed spike density functions.

We attempted to find support for the latency hypothesis using a number of latency methods. In fact, our basic FEF latency results (Table 2) were very similar to previous work from another laboratory (Pouget et al., 2005). This group used four other latency methods, almost all based on Poisson spike train analysis, and found no significant differences between the four methods. The fact that our population results fit nicely with their results, as well as with classic reports of FEF latencies (Mohler et al., 1973), suggests that latency measures are robust across laboratories, and largely independent of the specific latency metric employed.

Table 2. Latency of FEF visual responses.

Results in the top four white rows are latencies of first visual response onsets (correct trials only) for FEF data (n=90) in this dissertation. Results in the bottom four gray rows are from Pouget et al., 2005. “SD2” refers to time that activity reaches two standard deviations above baseline activity. “Peak” refers to time that activity reaches its peak during visual response. SDF = spike density function, followed by the width of the Gaussian used (5 or 10). “Max Diff” refers to a method that measures the time at which the visual response maximally differed from baseline activity, using a dynamic sliding window analysis (Sripati and Olson, 2009). This method was not used during any other analysis and shown for reference. Latency methods in gray are detailed in Pouget et al., 2005. Stars (*) indicate values that were limited by a fixed epoch size (50-150). Poiss. = Poisson.

method	mean	std. dev.	range: min.	range: max.
SD2, SDF_5	66	16	44	113
SD2, SDF_10	65	16	41	113
Peak, SDF_5	87	20	55	150
Peak, SDF_10	88	21	57	146
Max Diff	77	27	50*	150*
Poiss. spike train	64	19	29	118
Poiss spont. rate	73	33	3	152
Poiss. threshold	64	22	8	120
5% maximum	58	19	20	97

The latency hypothesis required that we measure the amount of time between visual responses. To do so, we first calculated the latency of first and second visual responses separately (Table 3). We found that second visual responses were accelerated in all brain areas relative to first visual responses; second responses started, peaked, and ended sooner than their first response counterparts. This could be because the initiation of second responses occurred while residual activity related to the first visual responses was not fully decayed. However, the difference between first and second responses was not simply a shift in response time relative to stimulus onset. Second visual responses were overall shorter in duration than first visual responses as well (Table 3, rightmost column). This effect was highly significant in all brain areas (Wilcoxon signed rank test on first versus second response durations, $p < 0.001$).

In sum, our preliminary analyses confirmed that visual response latencies in our task, and the methods used to detect them, were consistent with results from other animals, tasks, and laboratories. We therefore turned our attention to measuring the time between first and second visual responses in each trial to see if it encoded, in any way, the monkey's perceptual report of time interval.

Table 3. Visual response properties for 1st and 2nd responses to successive stimuli

Onsets, peaks, offsets, and durations of visual responses for two methods (5 or 10 ms kernel spike density functions). Values (ms) are means from correct trials. Shading segregates the columnar pairs that should be compared. In each columnar 1st-2nd response pair, the smaller value in each row is shown in bold. With one exception, 2nd visual responses were accelerated relative to 1st responses according to all measures, in all three brain areas. Also, as discussed in text, 2nd response durations were always shorter than 1st response durations.

Visual Response Property	<i>Onset</i>				<i>Peak</i>				<i>Offset</i>				<i>Duration (Offset-Onset)</i>			
SDF width	5ms		10 ms		5ms		10 ms		5 ms		10 ms		5 ms		10 ms	
Which response?	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
FEF	66	58	65	55	87	82	88	82	128	112	143	123	62	54	78	68
intSC	54	46	49	39	77	70	80	70	123	107	131	112	69	61	82	73
supSC	39	35	34	31	54	57	55	56	107	96	119	107	68	61	85	76

The combination of different latency measures used across three brain areas produced a large volume of results. Nearly all the findings were negative. In light of our positive results related to the magnitude hypothesis, however, we considered our negative results related to the latency hypothesis to be critical, and so we have tried to summarize them in a succinct manner. We summarize the latency analyses with combined table and figures. Figure 25 shows the first

latency data set. As detailed in the figure legend, the table contains the information regarding differences in correct versus incorrect inter-response timings in FEF, intSC, and supSC. The table includes mean inter-response time for correct and incorrect trials, the correlation coefficient, and the p-value of the correlation. (Note that the p-value in each cell is for the correlation. The p-value of the difference between correct and incorrect trials is indicated by the thickness of the cell's border line for rapid identification; see below). Because the latency hypothesis makes contrasting predictions for “short” and “long” time intervals, data are subdivided by interval group. To facilitate readability, statistical significance of the difference between correct and incorrect responses is indicated by thick lines around the cell containing the significant data. Thick red lines indicate significance *as predicted* by the latency hypothesis. Thick black lines indicate significance in the direction *opposite to* that predicted by the latency hypothesis. At a broad level then, if the latency hypothesis is true then we expect to see two thick red boxes side-by-side in the same row (for both “short” and “long” intervals). At the top of each table, we show two plots—the “short” and “long” raw FEF data using a 10-ms-wide Gaussian SDF (identical to the one used in the magnitude analyses above). We specifically designed this analysis and figure layout to be as analogous to the magnitude analysis as possible.

Time between first visual response onset and second visual response onset

We first analyzed the amount of time between visual response onsets (response onset asynchrony; ROA) using the SD2 metric. In FEF and intSC, we found no significant changes in response latency that fit with the latency hypothesis (longer ROAs in incorrect “short” trials and shorter ROAs in incorrect “long” trials). In fact, the only positive result for FEF contradicted the hypothesis. We found that ROAs in FEF were significantly longer in incorrect “long” trials than

in correct “long” trials (Wilcoxon signed rank test, $p = 0.04$). In this case, monkeys chose the “short” choice target even though the amount of time between visual responses *increased* relative to correct “long” responses. In supSC, ROAs were significantly elongated for “short” time intervals in support of the latency hypothesis ($p < 0.001$). There was no evidence of a corresponding change in the responses to “long” time intervals (Fig. 25; bottom row, “Long”).

We found no significant results when using the Peak latency metric on the same data (Fig. 26). Peak responses naturally occurred slightly later than the onset time to two standard deviations above baseline activity. In principle, it is possible that the few significant differences found in FEF and supSC using the SD2 metric to measure response onset are lost later when neuronal responses reach their peak firing rates. Regardless, unlike the complementary changes found for visual response magnitudes in both “short” and “long” SOA trials, we found no complementary changes in the time between visual response onsets and therefore little persuasive evidence for the latency hypothesis.

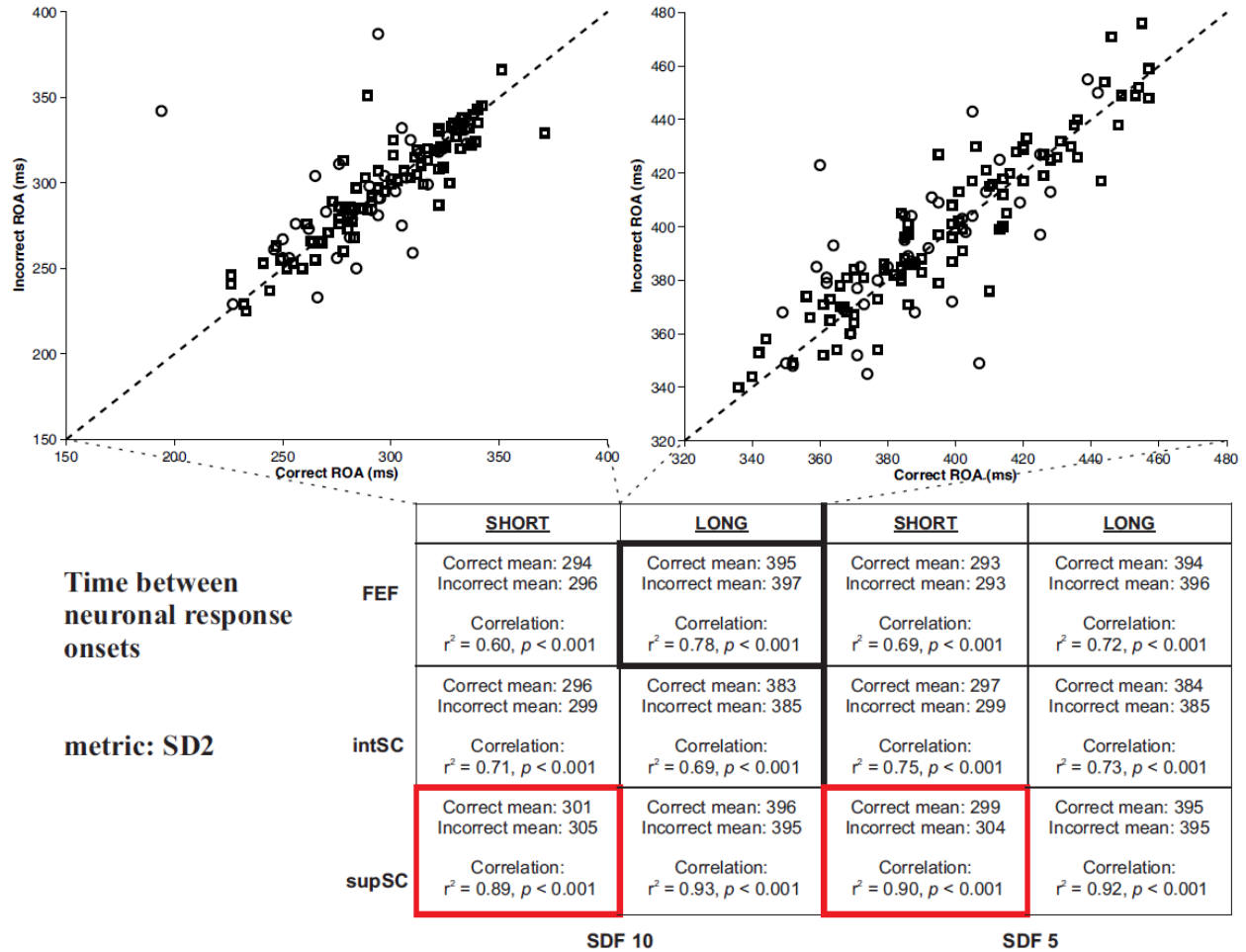


Figure 25. Amount of time between correct versus incorrect visual response onsets (ROA), using SD2 metric

Above, scatterplot of paired correct versus incorrect ROAs in FEF; “short” time intervals on left, “long” time intervals on right. Each symbol represents latencies at a single time interval for a given neuron; a single neuron can therefore have up to 2-3 points per plot. Below, table of mean, correlation coefficient and correlation p-value (not the correct vs. incorrect p-value). Cells are divided according to brain area (rows), width of spike density function (left two columns vs. right two columns), and time interval groups (columns within an SDF). Statistical significance of correct versus incorrect latencies was determined using a Wilcoxon signed rank test (a nonparametric paired t-test). Significance is indicated by thick outlines around a given cell ($p < 0.05$). Thin lines indicate p-values greater than 0.05. Thick black lines indicate significance in direction opposite of latency hypothesis; thick red lines indicate significance in direction predicted by latency hypothesis.

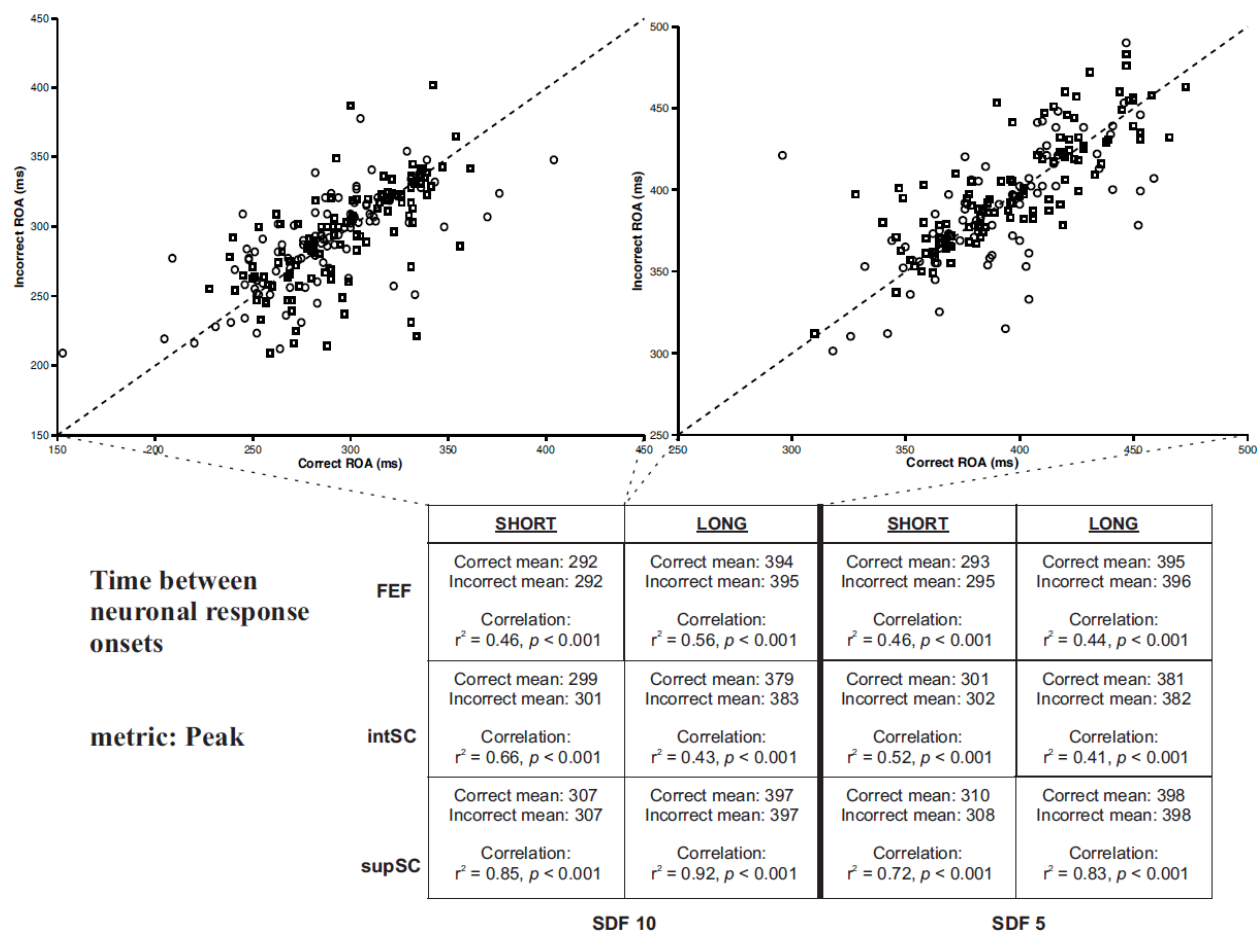


Figure 26. Amount of time between correct versus incorrect visual response onsets (ROA), using Peak metric

Same conventions as Figure 25.

*Time between first response onset and second response **offset**, and other response intervals*

Our task did not require the monkeys to use stimulus onsets to measure the intervening time between stimuli, nor can we be certain a priori that the brain should rely on the time between visual response *onsets*. In principle, any combination of onsets and offsets could be used as reference points to measure the presented time intervals: onset-onset, onset-offset, offset-onset, and offset-offset. Although referencing the two stimulus onsets (and corresponding neuronal response onsets) is arguably the most natural approach, for thoroughness we tested whether latency information was embedded in other response combinations. Of the remaining three possibilities, tracking the time between the first stimulus onset and the second stimulus offset (i.e., the duration of entire visual-related activity) seemed most likely. We consequently analyzed the time between the onset and offset of the neuronal responses.

We again found no consistent support for the latency hypothesis. Much of the data contradicted the hypothesis outright, and complementary changes in “short” and “long” time intervals were absent. Figure 27 displays differences in correct versus incorrect trials for “short” and “long” time intervals. We used the SD2 metric for measuring both the onset and offset of visual responses. The most robust finding for this particular metric was that response intervals (onset-offset) in supSC neurons were consistently longer in correct trials than in incorrect trials (Fig. 27, bottom row), regardless of SOA group (short or long). However, this effect was not robust across brain areas. In FEF neurons, there was no significant difference between correct and incorrect response intervals.

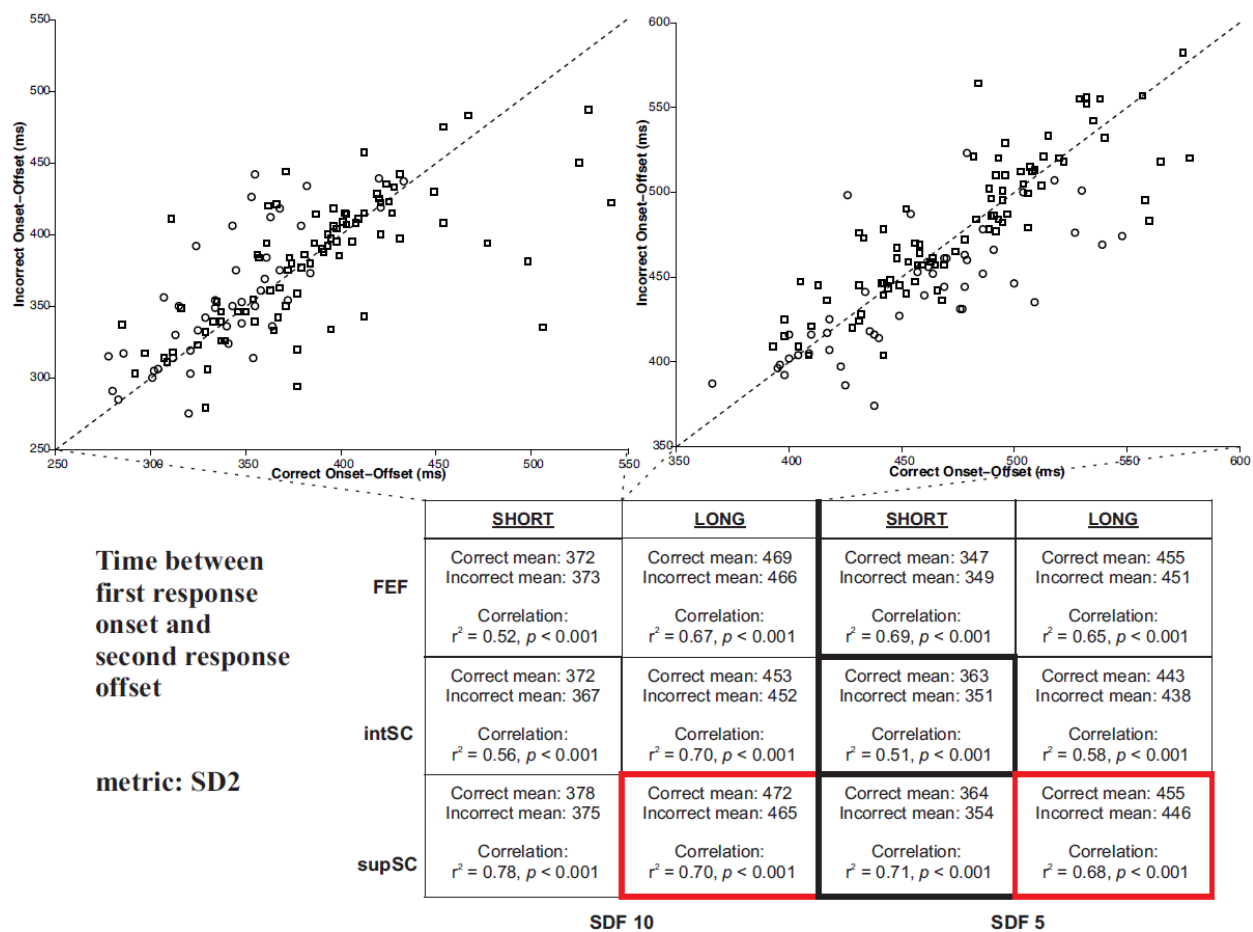


Figure 27. Amount of time between correct versus incorrect first visual response onset and second response offset, using SD2 metric

Same conventions as Figure 25.

We repeated this analysis process for all remaining inter-response possibilities (onset-offset, offset-onset and offset-offset), using both SD2 and Peak metric for detecting onset. We failed to find a single case of complementary change, in which “short” and “long” time intervals showed significant changes in opposing directions, which would be necessary for consistent latency encoding of time intervals.

Bootstrapped latency values

Neuronal latency measurements are sometime noisy and neurons themselves can show large variability. It was therefore possible that the results of our inter-response analyses were “contaminated” by highly variable neurons. To address this issue, we tested whether the more reliable neurons in our populations might provide more useful timing information regarding the monkeys’ choices. We did this in two ways. First, we replaced each neuron’s original latencies with the means of the resampled latency distributions of each condition (see Methods). Given random reshuffling of the original spike times, the mean of the resampled distribution may be considered a more reliable measure of the neuron’s response latency. After using the Peak metric to measure the time between response onsets (ROA), we plotted correct versus incorrect ROAs for “short” and “long” trials (Fig. 28). Compared with the original latency data (Fig. 26), mean shuffled latencies accounted for more of the variance. But significant changes in ROAs were largely unchanged. We found no significant differences using ROAs calculated from the original latencies. Here, we found a single significant difference in ROAs, between correct and incorrect ROAs in “long” trials. This difference was not significant for the same data convolved with a smaller Gaussian kernel. This analysis therefore provided some evidence to support the latency hypothesis, but it was not robust and it failed to account for behavioral performance in “short” time intervals.

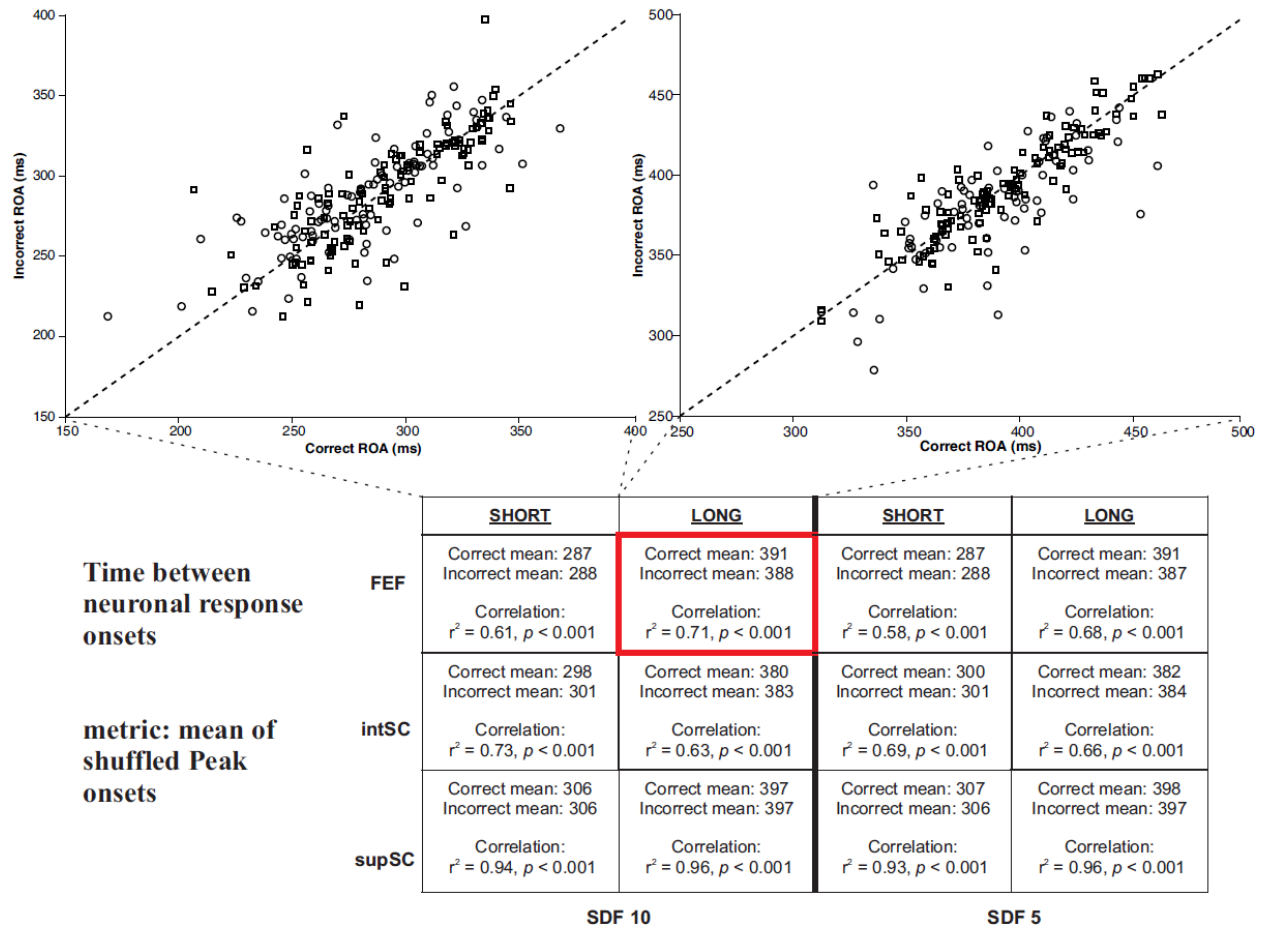


Figure 28. Amount of time between correct versus incorrect visual response onsets (ROA), using mean of shuffled Peak distribution metric

Compare to original latency values in Figure 26. Same conventions as Figure 25.

Second, we also performed the same analysis using only our most reliable neurons, as measured by the standard deviation of the neurons' resampled latency distributions (see Methods). For each area, we looked at the half of our neurons (the top 50%) with the most reliable visual response onsets (Fig. 29). Our results mirrored what we found when using the mean of the shuffled distributions. FEF ROAs were significantly longer in correct "long" trials

when compared to those of incorrect “long” trials, as predicted by the latency hypothesis. But again we did not see a significant difference in “short” trials in any of the brain areas.

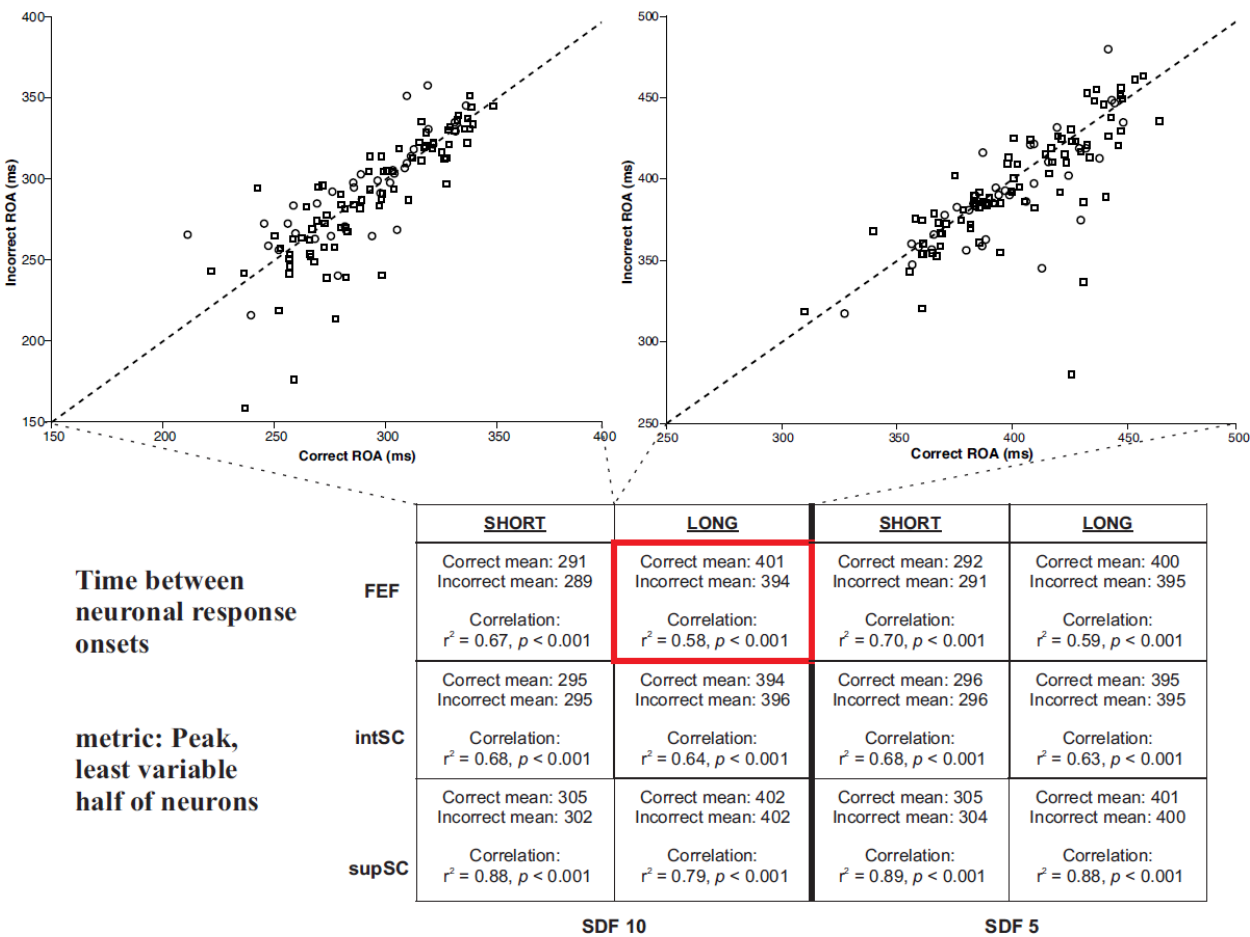


Figure 29. Amount of time between correct versus incorrect visual response onsets (ROA), using Peak metric on more reliable 50% of each population

Same conventions as Figure 25.

To confirm that our analysis method was sensitive enough to detect potential latency changes, we compared correct population ROAs from two adjoining time intervals instead of our usual correct versus incorrect comparison within the same time interval. In principle, these two

populations should differ by ~17 ms, which is also approximately the amount of time visual responses might differ if the monkey incorrectly mistook one interval for another. Tests of the amount of time from first visual response onset to second response onset between adjoining correct ROAs (e.g., 317 and 333 ms) were highly significant (rank sum test, $p < 0.001$), indicating that our lack of support for the latency hypothesis was not due to a lack of sensitivity in our analyses.

Finally, we looked exclusively at the time intervals surrounding the reference interval (SOAs of 333 and 367 ms). We reasoned that the latency variations at these difficult time intervals were most likely to elicit incorrect choices because relatively small latency changes could “push” the interval closer to or to the opposite side of the reference interval. However, even after looking at these time intervals in isolation, we found no significant change in the amount of time between visual responses as a function of task performance (e.g., onset-onset using SD2 metric and SDF = 10 ms, Wilcoxon signed rank test, SOA 333: $p = 0.53$, SOA 367 $p = 0.88$).

Overall, we found that no single latency metric fulfilled the predictions of the latency hypothesis for both “short” and “long” time intervals. While it is possible that the visual system relies on a more complicated combination of mechanisms, our analyses suggest that it does not rely solely on the latency of visual responses to keep track of the duration of events in the world.

3.4.6 Effect of motor activity on FEF magnitude encoding of time intervals

In our time interval discrimination task, choice target locations were fixed across trials during a recording session (see Methods). We tried randomizing the choice target locations during

training but it proved too difficult for the monkeys, even after ~4-6 months of exposure. In order to move forward with the experiment, we positioned the choice targets at the same location on every trial. Consequently, potential selectivity for either a short or long time interval was confounded with selectivity for the movement to the associated choice target. It was therefore possible that our reported changes in visual response magnitude were related, at least in part, to saccade generation toward one of the two choice targets.

We took several precautions to minimize the effects of motor and premotor activity. First, choice targets were always placed well away (on average $\sim 15^\circ$) from the neuron's RF and in the visual hemifield ipsilateral to physiological recordings. Second, we reversed the response mapping between monkeys (see Methods). Third, we only recorded from visually-responsive neurons, which likely did not eliminate saccade responses entirely but at least biased our population sample away from purely saccade-related cells (see Figure 30 below). Finally, we recorded from both FEF and intSC, two closely-linked visuomotor structures with comparable response properties (Mohler and Wurtz, 1977; Sommer and Wurtz, 2004). If saccade-related artifacts were found in one structure, it seemed likely that we would find similar effects in the other structure.

In light of these factors, the possibility of significant premotor effects was unlikely. Neurons needed to show selectivity for a target location (one of the choice targets) outside of its RF and always located in the ipsilateral hemifield, where neurons are generally less responsive. Because magnitude changes were apparent at the time of the second visual response (Fig. 17), putative premotor activity needed to begin during the visual epoch and prior to knowing which choice target to select (i.e., where to plan an eye movement to). If such activity existed, it needed to fire more strongly in response to the “long” choice target relative to “short” choice

target, the latter $\sim 12^\circ$ degrees away and also located in the ipsilateral visual field. If activity fulfilled these criteria, it finally needed to manifest itself as a preference for opposite choice target locations for the two monkeys (greater FR for the lower choice target in monkey K, and greater FR for the upper choice target in monkey C) due to our reversed response mapping. There is little support for such selectivity given these constraints. There is limited evidence of ipsilateral responsiveness in lateral intraparietal sulcus (LIP; Heiser and Colby, 2006; Freedman and Assad, 2009) but that, if applicable to FEF, only accounts for one of the requirements above. Nevertheless we carried out additional tests to ensure that our results were not caused by premotor or motor activity. Because our main result pertains to significant magnitude changes in FEF, we limit our discussion below to this population of neurons.

When stable neuronal isolation allowed, we recorded FEF responses during two additional tasks. First, we recorded neuronal responses ($n=16$) outside the context of the time discrimination task but using the same fixation and saccade locations. Monkeys maintained fixation and then made a saccade to one of two choice target locations. The initial fixation point in the control task was identical to the fixation location used during interval presentation in the time interval discrimination task. Saccade targets were also placed at locations identical to those used in the discrimination task. On each trial, one of the two saccade targets was illuminated and the monkey was rewarded for making an eye movement to that location. If our magnitude results were simply due to changes in activity based on the impending saccade, we would expect to see significantly greater firing rates for saccades to the “long” choice target location. Yet we only found that 12.5% (2/16) of FEF neurons tested showed such a differential effect (rank sum test, $p < 0.05$), far below the 42% of FEF neurons that showed significant activity in our time

interval discrimination task. Results from this control task therefore provided minimal support, at best, for the role of (pre)motor activity in our magnitude results.

We also recorded from a majority of our FEF neurons ($n = 46$) during a memory-guided saccade task (detailed in Chapter 2). We measured activity during visual (V) and saccade (motor response M) epochs in this task to rank each neuron using a “visuomotor index” ($VMI = V - M / V + M$) and quantify the respective response components (Bruce and Goldberg, 1985; Lawrence et al., 2005). Briefly, the greater the VMI, the stronger the visual activity compared with the saccade-related activity. Figure 30 (top) shows that our population of FEF neurons was in fact biased towards visually-responsive neurons (mean = 0.32, 1-sample t-test, $p < 0.001$). Therefore our population results in general may not necessarily be representative of all FEF neurons. FEF neurons with significant CPs (21/46) showed a range of VMI values (Fig. 30 top, filled bars), indicating that magnitude changes were not strictly limited to motor-dominant neurons but found in visually-dominant neurons as well. A closer look at the FEF neurons with significant grand CPs revealed that although there were an equal number of visual- and saccade-related neurons with CPs greater than 0.5 ($n = 8$ for each), significant grand CPs less than 0.5 (opposite to the direction predicted by the magnitude hypothesis) were exclusively visual ($n=4$; Fig. 30, bottom). This hints that choice-related activity may be encoded differently in FEF neurons based on a neuron’s basic visuomotor properties, but our sample size is too small to meaningfully make such a claim. In sum, visual response magnitude changes were evenly distributed across FEF neurons regardless of the neurons’ relative strength of saccade-related activity.

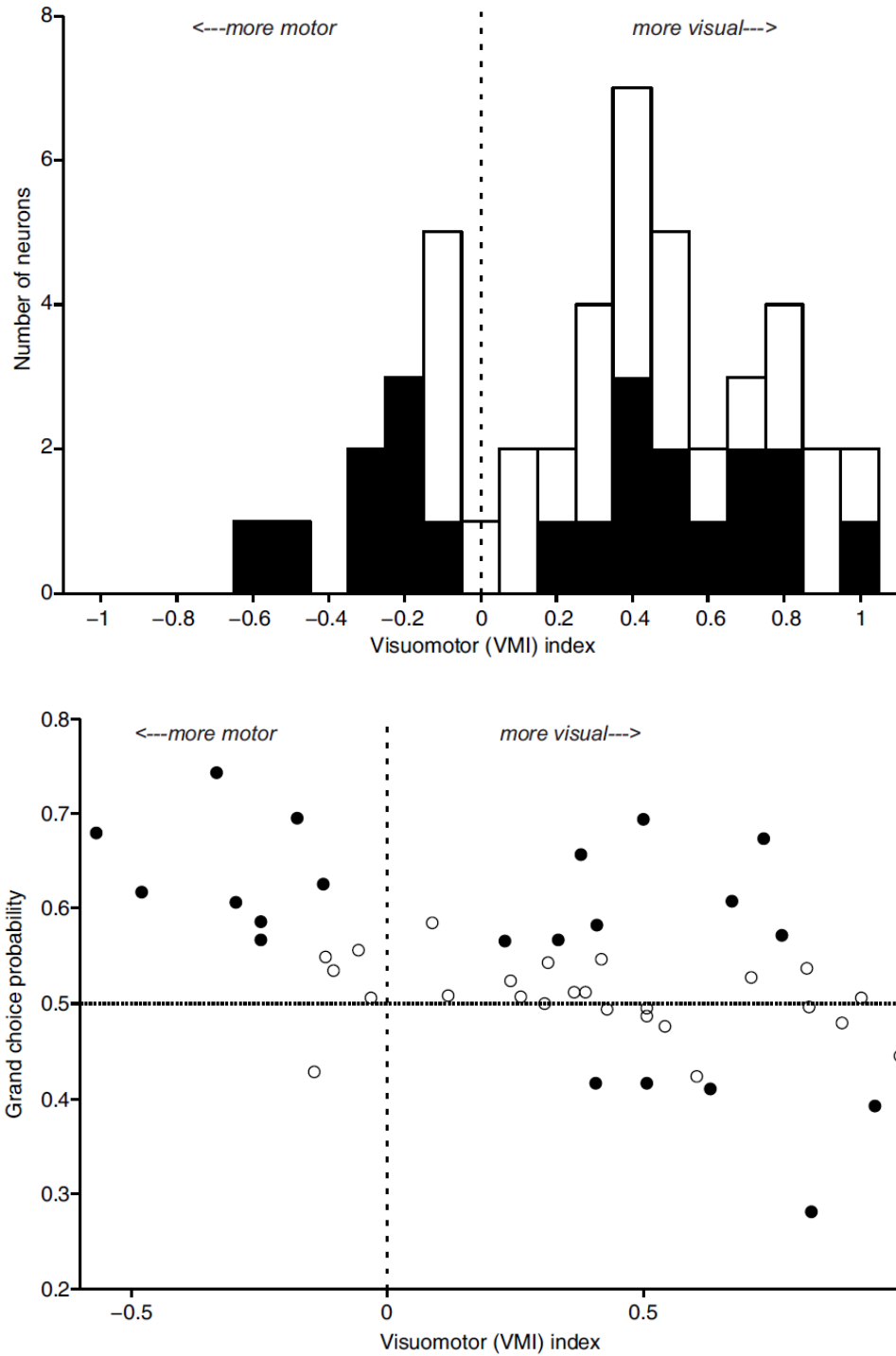


Figure 30. Choice probability and visuomotor properties of FEF neurons

Top, Visuomotor index (VMI) values of FEF neurons (n=46). VMI of 1 indicates a purely visual neuron with zero saccade related activity, while a VMI of -1 indicates a purely saccadic neuron with no visual activity. Filled black bars indicate neurons with significant CPs (n=21). *Bottom*, VMI of the same FEF neurons plotted as a function of their grand CP values. Filled circles represent neurons with significant grand CPs.

As a final test for the effect of motor activity, we looked at FEF visual responses in the time interval discrimination during trials in which the reference interval (350 ms) was presented. These trials provided an ideal test for potential motor influences. The visual stimuli were ambiguous—not clearly “short” or “long”—and therefore monkeys made nearly the same number of saccades to both choice targets (Fig. 12); trials otherwise contained saccade parameters identical to those in non-reference trials, but still within the context of the time interval discrimination task. We sorted the reference interval trials according to saccade location for each monkey, correcting for the reverse choice target mapping between monkeys. We found no differences between the firing rates of first visual responses, as expected (Fig. 31, top). The same analysis on second visual responses—where magnitude changes occurred at other time interval—also revealed no difference in FEF firing rates as a function of impending saccade target (Fig. 31, bottom; Wilcoxon signed rank test, first response: $p = 0.48$; second response: $p = 0.99$).

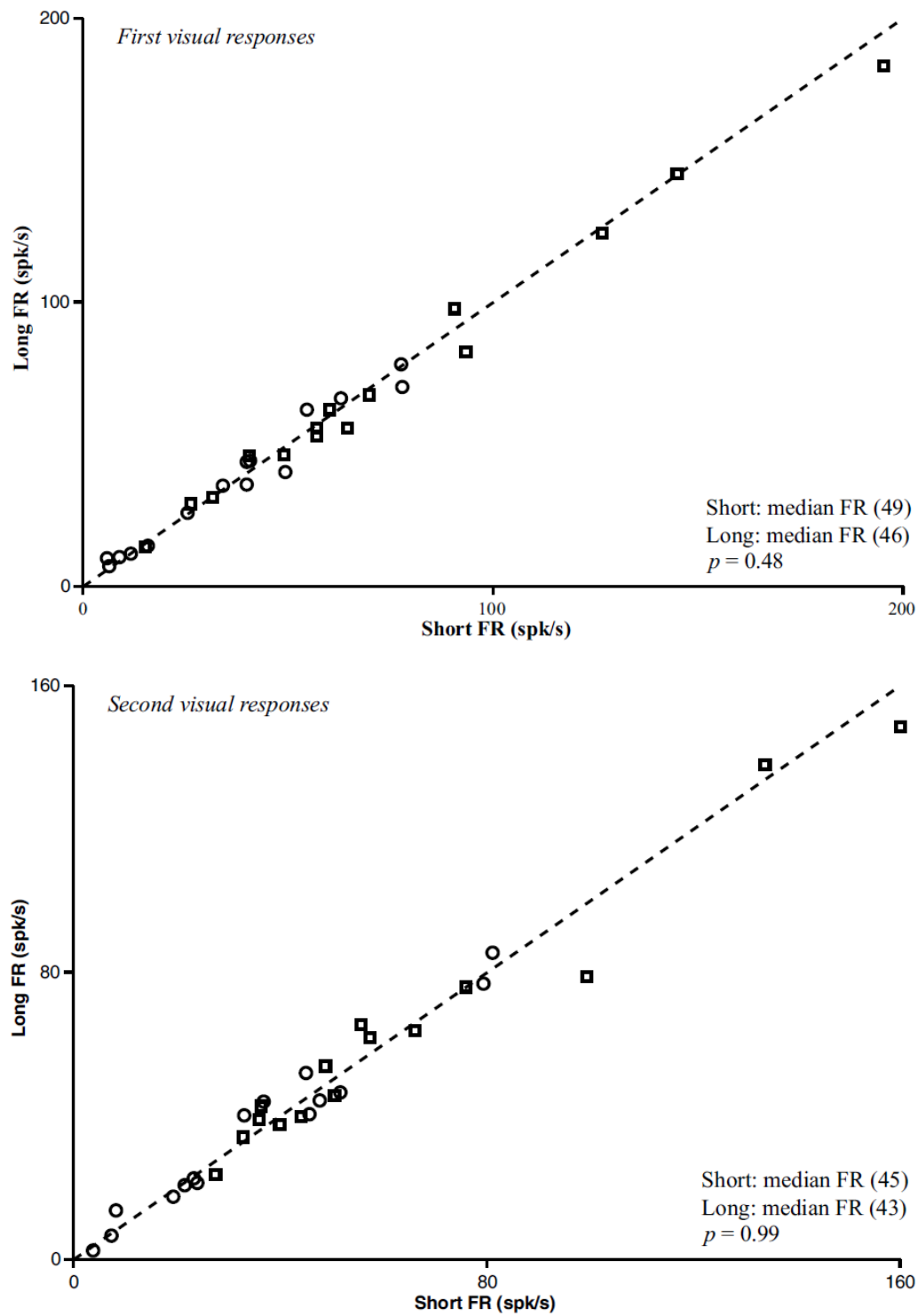


Figure 31. No significant changes in neuronal activity as a function of saccade direction during reference interval trials

Top, first visual responses. *Bottom*, second visual responses. $N = 30$ (15 neurons from each monkey).

Lastly, previous work in other cortical regions illustrated the influence of small ($\sim 2\text{-}3^\circ$) eye movements, termed “microsaccades”, on choice probability during motion detection tasks (Herrington et al., 2009). Microsaccades, like standard saccades, have been shown to suppress visual activity, including in intSC (Nienborg and Cumming, 2006; Herrington et al., 2009; Hafed and Krauzlis, 2010). Consequently, for microsaccades to be a confounding motor factor in our task, they needed to occur disproportionately more during “short” correct trials (compared to incorrect trials) and “long” incorrect trials (compared to correct trials). Such a finding would, for example, account for the diminished activity in “short” correct trials relative to “short” incorrect trials in Figure 17. For each monkey, we calculated the rate of microsaccades during the fixation epoch surrounding the time of interval presentation for all trials. Both monkeys showed significant differences in their rates of microsaccades, but neither showed changes that could account for our magnitude results. Monkey K showed no significant difference in “short” trials (rank sum test, $p = 0.5$), but made significantly more microsaccades during correct “long” trials when compared to incorrect “long” trials (rank sum test, $p < 0.001$). Monkey C showed significantly more microsaccades on incorrect “short” trials compared to correct “short” trials (rank sum test, $p = 0.02$), but no difference in “long” trials ($p = 0.23$). Thus, suppressive effects due to microsaccades are an unlikely explanation for the changes in response magnitude seen between correct and incorrect trials.

3.4.7 Role of delay activity in magnitude changes

Our population plots hinted at the possibility that changes in magnitude might occur before the second visual response, during the interstimulus interval. To investigate this possibility, we again focused our analysis on FEF neurons and quantified the amount of delay activity in each. We measured the amount of activity 100 ms before second stimulus onset and compared it to the baseline firing rate. We made this measurement only for time intervals longer than 300 ms to safely exclude the offset of the preceding first visual response in shorter SOAs, and to allow for a full 100 ms delay epoch for all responses. Nearly one-third of FEF neurons showed no significant delay activity (29/90). The remaining ~68% of FEF neurons showed significant delay activity in at least one time interval (61/90), consistent with the prevalence of delay activity found in other tasks (Sommer and Wurtz, 2001; Wurtz et al., 2001). Delay activity was present at various degrees across multiple time intervals in the same neuron. Some neurons only showed significant delay activity in one out of five time intervals tested, while others showed delay activity in five out of six intervals tested. In total, 35 (out of 90) neurons showed significant delay activity in 50% or more of their tested time intervals. However, only one neuron showed significant delay activity in all of its time intervals.

We were interested in whether delay activity played a significant role in the magnitude changes seen around the time of the second visual responses. To investigate this potential effect, we pooled the time intervals with significant delay activity for each FEF neuron. This process yielded 98 data points, on average about three time intervals from each delay neuron. As a measure of the strength of delay activity, we used the p-value obtained from the initial delay activity versus baseline activity comparison (Wilcoxon signed rank test). We compared this “delay strength” value with the neuron’s CP for the same time interval. We found no significant

correlation between strength of delay activity and CP ($r^2 = 0.10$, $p = 0.33$). This result indicates that the magnitude changes, as measured by CP, were not correlated with the amount of delay activity across our population of FEF neurons.

3.5 DISCUSSION

We tested the idea that the time code implicit in neuronal adaptation might be used by the brain to represent visual time intervals. This “magnitude hypothesis” predicts that the time between two visual events is represented by the strength of the second visual response, with longer time intervals associated with larger responses. We compared this hypothesis with a more intuitive hypothesis in which the time interval is simply demarcated by the time between the first and second visual response. To test these hypotheses, we recorded from visually-responsive neurons in FEF, intSC, and supSC while monkeys performed a task that required them to discriminate the amount of time between two stimuli. Our analyses focused on differences in neuronal activity when the monkey correctly versus incorrectly judged the amount of time between the same exact visual input (a particular time interval).

Our central finding was that single neurons in FEF showed systematic changes in second visual response magnitude relative to the monkeys’ perceptual reports. The direction of these magnitude changes (smaller responses for shorter intervals, and larger responses for longer intervals) matched the direction of changes seen during neuronal adaptation. For time intervals shorter than the reference interval, visual responses were stronger in incorrect trials (judged as “long”) than in correct trials (judged as “short”). Conversely, for “long” time intervals, visual

responses were stronger in correct trials (judged as “long”) than in incorrect trials (judged as “short”). These complementary patterns of magnitude changes were not an inevitable result. A priori, it seemed likely that magnitude changes would manifest as decreases in activity on all incorrect trials regardless of the presented time interval (Cook and Maunsell, 2002). Diminished activity, along with decreases in behavioral performance, is a hallmark of “misallocated” spatial attention. While spatial attention likely played a key role in our task (for example, by focusing neural resources on the time of stimulus onsets), attention by itself cannot account for our reported magnitude changes. Specifically, aberrations in spatial attention would not predict a relative *increase* in the size of second visual responses during incorrect “short” trials.

We found no support for the magnitude hypothesis, however, in intSC and supSC neurons. While this result is not entirely surprising for retinal-recipient supSC, we would not have predicted such a result for intSC. Previous studies have found significant choice-related activity in intSC, albeit in different task conditions (Krauzlis and Dill, 2002; Horwitz et al., 2004). Our data support the notion that a small minority of intSC neurons may represent an “abstract perceptual decision” (Horwitz et al., 2004), now expanding that definition to include temporal information. Our proportion of intSC neurons with significant CP values (11%) matches the previous estimate of Horwitz et al. (2004). But despite the small number of neurons with significant CPs, intSC neurons as a population did not seem to accumulate choice-related information as the task progressed, especially when compared to FEF (Fig. 24). Alongside recent work highlighting the higher cognitive capabilities of intSC (Lovejoy and Krauzlis, 2010), the SC therefore appears to play a small role in decision making, at least in the context of temporal judgments. It remains to be determined whether this role is in instrumental or simply ancillary.

Monkeys performed the task accurately and showed a monotonic, sigmoid-shaped increase in “long” choices as the intervals increased in duration. However, we note that we found that untrained human subjects could perform at levels equivalent to the monkeys in a single training session (data not shown). We therefore interpret the long training time required for monkeys as the time needed to nonverbally communicate the parameters and instructions for the task. The fact that our task was demanding but well within the range of human abilities supports the notion that our results are applicable to temporal discriminations at behaviorally relevant, naturalistic timescales.

Population activity measures and cumulative CP analysis revealed that differential activity related to the animal’s perceptual report of the time between two stimuli emerged after the first visual response, around the time of the second visual response. These results are compatible with the task requirements, where accumulation of timing information needed to start after the first stimulus but a decision about the correct response could not be made until the second visual stimulus (in “short” trials) or, at a minimum, until the time of the reference interval (in “long” trials). In this way, FEF neurons acted like ideal observers, transforming the time intervals into putative choice-related activity in a rapid amount of time. We are still developing a neuron-by-neuron method of analyzing this further. But we can currently estimate the time course of temporal decision making from the population data. For “short” trials, the average weighted SOA (mean of 250, 267, 283, 300, 317, and 333) was ~300 ms, after accounting for more trials closer to the reference interval. This means that, on average, the second visual stimulus turned on 300 ms after first stimulus onset. Our latency analyses above indicate that it takes ~65 ms for the second stimulus to evoke a visual response in FEF. Strikingly, in our

population difference signals we see a significant deviation at ~370 ms in the population of “short” trials, nearly equal to the estimated $300 + 65$ ms.

It is remarkable that the difference in activity at this time point remains significantly different from “baseline” (no difference between correct and incorrect trials) for the remainder of the trials. This putative rapid decision-making activity fits with recent results in FEF that show reliable neuronal discrimination in as little as 30 ms (Stanford et al., 2010). Our task encouraged monkeys to deliberate before making a choice because we wanted to establish the potential sensory changes involved with time interval encoding. However, follow-up work could push the limits of perceptual processing and behavior by requiring the monkeys to make rapid choices. Such an approach would begin to dissociate some of the possible factors involved in our decision signal. Therefore more experiments are needed to understand the precise nature of the information contained in putative choice-related activity and its usefulness for behavior.

We used a variety of approaches to measure the time between visual response latencies, yet they all yielded limited support, at best, for the latency hypothesis. Although we occasionally found changes for either “short” or “long” time intervals consistent with the latency hypothesis, we also found changes in the opposite direction. Critically, we were unable to find any complementary changes for both sets of time intervals as the latency hypothesis required. One caveat is that our analyses of the latency hypothesis assumed that “time in the world” is equivalent to “time in the brain”; for example, that a second visual response from in an incorrect “short” SOA trial might occur 5-10 ms later than usual, thereby explaining the “long” choice. But this direct mapping between “world time” and “brain time” is not necessarily the case, especially at short timescales. It is possible that small fluctuations in neuronal latencies have

large effects on perceived time. If true, tiny but meaningful latency changes may have gone undetected in our analyses.

Another caveat is that time perception at naturalistic timescales may not be encoded in a way that is readily observable at the level of serially-recorded single neurons, like some neural signatures of attention (Cohen and Maunsell, 2010). Inter-neuronal or system wide representations distributed across neurons may provide more accurate views of time perception in the brain (e.g., Mauk and Buonomano, 2004). If this is the case, a promising solution is simultaneous multi-electrode recordings, which would allow researchers to look at inter-neuronal correlations. It should also be noted that, in principle, these caveats apply just as much to magnitude coding. Nonetheless, we did find a robust code for time intervals in the strength of visual activity. It is perhaps the case that the apparent magnitude code works together with a more subtle latency code to represent brief time intervals. However, based on our results, we conclude that the magnitude hypothesis is a better description of neuronal time discrimination at brief timescales than the latency hypothesis.

The magnitude encoding of time intervals suggests that the brain does not need to encode the timing of events in the world solely by the timing of associated neuronal responses. Instead, the brain may refer to non-temporal dimensions, i.e., the size of visual responses. Although we were (to our knowledge) the first to test this idea in single neurons, magnitude-related encoding of time has received increasing support in the past few years (Grande and Spain, 2005; Noguchi and Kakigi, 2006; Pariyadath and Eagleman, 2007). We used two *identical* stimuli and correlated neuronal activity with opposing behavioral reports (correct and incorrect time judgments). But *differing* stimuli could also be used in more causal experiments to directly manipulate the strength of visual responses. Studies that manipulate the (presumed) size of

visual activity elicit strong time illusions, overwhelmingly supporting the magnitude hypothesis (for review, see Eagleman and Pariyadath, 2009). The magnitude hypothesis may also help explain why human time perception is so malleable (see Introduction). However, it also leaves open the question of what “safety” mechanisms are in place to prevent time perception from becoming a continuous stream of saccade-induced time illusions (see Chapter 4 for further discussion).

Our results are premised on the fact magnitude encoding should take place on the same timescale as neuronal adaptation, but we did not explicitly demonstrate that adaptation was required for changes in magnitude to take place. As a result, we cannot be certain that adapted visual responses are directly referenced as a source of timing information. In fact, there are reasons to suggest that this is not the case. First, in some of our analyses, timing information between SOAs within the “short” or “long” group did not significantly differ. These analyses suggest that changes in magnitude may not be precise enough to distinguish a time interval of, say, 300 ms from a time interval of 333 ms. In this sense, FEF neurons may be integrating and solely representing the “choice” of upstream sensory neurons, or even from putative timekeeping structures like the cerebellum or basal ganglia. Future work is needed to investigate the role of neuronal adaptation at intermediate stages of visual processing and as well as their contribution to time interval discrimination.

Finally, we cannot completely discount the possibility that errors in time judgment were related to the storage and recall of the stored reference interval, as opposed to simple changes in stimulus encoding. On each trial, monkeys were required to compare the SOA of the presented time interval with the SOA of a learned reference interval (350 ms). Therefore the activity of FEF neurons could reflect some sort of categorical processing between “short” and “long” SOAs

with the reference interval as the categorical boundary (Ferrera et al., 2009). However, such a coarse-coding explanation does not readily explain why the monkeys performed in such a finely discriminatory manner. The behavioral performance was not suggestive of categorization, which would reveal itself as flat performance on either side of a sharp deflection at a boundary (see Chapter 4 for further discussion). In contrast, our monkeys produced smooth, gradually-changing performance curves as a function of SOA.

On the other hand, a categorization hypothesis could perhaps account for our negative latency results. It is possible that presented time intervals were accurately represented by visual response latencies, but incorrect trials contained “problems” (whatever that entails) with recollection of the reference interval unrelated to response latencies. We think this is highly unlikely, however, because as in most decision-related animal studies, our monkeys were highly overtrained. Training on the task took approximately 6 months to a year, and recording happened after that extensive training period. For these reasons, we are reasonably confident that our results are not due to time interval-dependent changes in reference interval storage and retrieval during categorization.

In conclusion, we showed that the magnitude of visual responses in FEF, but not in SC, correlates with the discrimination of sub-second time intervals. The directions of the magnitude changes were predicted by the referring to the implicit temporal code embedded in neuronal adaptation. Surprisingly, variations in visual response latencies did not correlate with variations in time interval judgments. Our findings provide new insight into the neuronal mechanisms of time perception within the larger context of sensory processing and decision-making.

4.0 GENERAL DISCUSSION

An overarching goal of this dissertation was to provide a neurophysiological framework for investigating visual time perception at behaviorally relevant timescales. We recorded from single neurons in FEF (Chapters 2 and 3), intSC (Chapter 3), and supSC (Chapters 2 and 3). We measured the strength and time course of neuronal adaptation during passive fixation in FEF and compared them with those in supSC (Chapter 2). Second visual responses were strongly suppressed in both areas if sequential stimuli were separated by less than ~200 ms. Recovery of the second visual response increased as the interstimulus interval was lengthened, with full recovery for intervals greater than ~500 ms. Overall, the amount of suppression caused by repeated visual stimulation was greater in FEF than in supSC, consistent with other studies of adaptation between brain areas (Verhoef et al., 2008). These foundational results measures support the claim that adaptation is robust throughout the visual system, from the midbrain to prefrontal cortex.

We next trained monkeys to report their perception of time intervals. We used time intervals at an intermediate range of neuronal adaptation, where second responses were readily detectable but not so obscured as to hinder analysis. We tested the idea that neuronal adaptation contains useful, implicit timing information in the strength of the second visual responses. If this “magnitude hypothesis” were true, then we expected to see changes in the *size* of second visual responses that correlated with perceptual reports, not simply changes in *latency* (“latency

hypothesis”). We found, in fact, that changes in the magnitude of second visual responses accounted for the monkeys’ behavioral choices better than changes in the latencies of visual responses. Incorrectly reported time intervals elicited visual responses that made sense in the context of neuronal adaptation; incorrect “short” trials had larger second visual responses (representing a longer passage of time), and incorrect “long” trials had smaller responses (representing a shorter passage of time). This magnitude coding of time intervals was present only in FEF neurons; SC neurons showed no significant changes in response magnitude or latency that correlated with perception.

In this chapter, we highlight future directions, expand upon discussion points from Chapter 3, and review the implications of adaptation as a putative neuronal timekeeping mechanism.

4.1 VISUAL FLUTTER: MANIPULATING THE MAGNITUDE HYPOTHESIS

Our time interval discrimination task was, in many ways, analogous to seminal work from Romo and colleagues on frequency discrimination in the somatosensory system (Mountcastle et al., 1990; Romo et al., 1993). Romo et al. trained monkeys to discriminate sinusoidal pulse frequencies that caused small indentations on the surface of the monkeys’ fingertips. The researchers then measured corresponding changes in neuronal activity in primary sensory cortex and other areas (e.g., de Lafuente and Romo, 2006). Romo et al. called this task “flutter discrimination” (Romo and Salinas, 2003), referring to the physical sensation on the fingertip. The flashes in our task could be considered “visual flutter”, as both flashes stimulate the RF of

an individual neuron. The work of Romo and colleagues therefore provides a potential roadmap for time interval discrimination in the visual system.

Two manipulations from the progression of work by Romo et al. are particularly amenable to building on the results presented in this dissertation (Romo et al., 2000; Brody et al., 2002). First, one could expand on the correlational experiments performed here by performing causal tests of magnitude hypothesis. One approach would be to artificially augment—and perhaps eventually replace—the second visual stimulus with microstimulation (inspired by Romo et al., 2000). In its simplest form, this experiment would involve small, sub-threshold injections of current during the presentation of the second visual stimulus on randomized trials. If the magnitude hypothesis holds, then it should be possible to titrate the microstimulation so that small increases in amplitude yield an increase in “long” time interval percepts. Microstimulation could also be used in an analogous way to test the latency hypothesis. The timing of visual stimulus onsets is somewhat limited by the refresh rate of video hardware, but microstimulation can be applied with millisecond precision. Thus, instead of trying to simply increase the strength of the second visual response, we could alternatively attempt to “push” the visual response earlier/later by adding spikes (in the form of microstimulation current) at the beginning/end of the visual stimulus presentation. One could perform such experiments with even more temporal precision using fast-response visual stimulus systems (e.g. lasers or LEDs). Although theoretically exciting, any use of microstimulation requires careful parameterization and rigorous controls for perceptual artifacts unrelated to visual stimulation in the RF (Murphey et al., 2009; Ni and Maunsell, 2010). Also, interpretations of microstimulation effects must always be tempered by the consideration that fibers of passage are activated in addition to sparse, local neuron cell bodies (Histed et al., 2009). Nonetheless, this first, simplest experiment would

be likely to yield relatively rapid and informative results about the plausibility of the magnitude hypothesis.

A second manipulation, also incorporated by Romo et al. (cf., Romo et al., 1997; Romo et al., 2002), would be psychophysical. One could convert the paradigm we used from a categorical-response task to a serially-presented, explicit two interval time comparison task. The latter would involve a continuum of short-long comparisons rather than fixing all comparisons to a learned short-long boundary SOA. We initially tried such a task on one monkey but the animal could not seem to learn it. It seemed to us that the monkey had trouble segmenting the beginning and end points of paired time interval stimuli embedded in a sequence of four stimuli. As a result, we designed our task to be more straightforward, with a single reference SOA. If monkeys could eventually learn an explicit two-interval task, it could help clarify the impact of putative “category-selective” neurons, if any (see below). It would also allow us to readily test the flexibility of the magnitude hypothesis at different timescales. On each trial, we could simply change the reference interval from, say 350 ms as we did in Chapter 3, to 1000 ms, where we see no neuronal adaptation in FEF or SC. In this way, we could determine if the magnitude hypothesis is limited to the timescale defined by neuronal adaptation.

Inactivation is a third possible manipulation (unrelated to Romo et al.). However, in the context of testing the magnitude hypothesis, this approach would involve the reduction of the second visual response to see if time intervals were perceived as shorter. Current methods do not permit such temporally precise activity reduction or elimination, although methods on the horizon could overcome this problem (Diester et al., 2011).

4.2 TEMPORAL CATEGORIZATION

The discovery of categorical neurons (Freedman et al., 2001) raises interesting questions about the neural underpinnings of decision making in our task. As mentioned in Chapter 3, our task required categorical responses. Monkeys viewed a range of SOAs but then had to classify them in only two ways, as “short” or “long” relative to the reference interval. Behavioral performance in other category-specific tasks have shown higher, more step-like performance on either side of the decision boundary (Freedman et al., 2001; Freedman and Assad, 2006), in contrast to the sigmoid functions that we found (Fig. 12). Yet we cannot rule out, based on our behavioral data, the possibility that FEF neurons were performing categorization. The extreme SOAs (i.e., 250 and 450 ms) could have served as category exemplars, as the canonical “cat” and “dog” in the original Freedman et al. experiment (2001). Such a hypothesis implies that categorization signals should be stronger at more extreme SOAs, but that is not what we found (Fig. 21). Given the small number of papers on the topic ($n \approx 6$), and their limited recording sites in LIP, MT and dIPFC, we cannot disentangle these possibilities at this time. Furthermore, it is equally plausible that a “perceptual decision [signals] and categorical signals are one and the same” (Freedman and Assad, 2011), thereby calling into question the utility of delving into the decision versus category dichotomy at all.

Crucially, even if our FEF neurons displayed categorization-like activity rather than fine-grained temporal discrimination, our results still provide a significant contribution to the field for three reasons. First, this would be one of the first reports of category-selective neurons in FEF (only other is Ferrera et al., 2009). Second, this would be the first demonstration of *temporal* categorization in single neurons. Previous studies looked only at the categorization in the spatial domain, e.g., shapes (dogs vs. cats; Freedman et al., 2001) or directions of motion (left vs. right;

Freedman and Assad, 2006). Expanding categorization to include the temporal domain opens up new possibilities regarding what category-like neuronal responses might be used for and how they are related across brain areas. Finally, categorization followed the changes in firing rate predicted by the magnitude hypothesis; “long” categories displayed larger second visual responses relative to the identical stimuli presented and perceived as “short”. This suggests, for the first time, that the categorization functions of neurons and their underlying neuronal adaptation properties interact. Moreover, this interaction may be the first evidence of a guiding principle defining—at a conceptual if not mechanistic level—*why* the firing rate for Category A increases while that of Category B decreases, as opposed to vice versa.

4.3 ADAPTATION AS A TIME KEEPING MECHANISM

We demonstrated above that rapid consecutive visual stimulation leads to a robust first neuronal response followed by smaller subsequent responses that recover as a function of the length of the time between stimuli (neuronal adaptation). Despite our understanding of the physiology of adaptation (see Chapter 1), its role in conscious perception is poorly understood. However, results from Chapters 2 and 3 provide evidence for a critical, previously-overlooked feature of neuronal adaptation; it contains reliable temporal information, with the sizes of consecutive sensory responses encoding the amount of elapsed time between stimuli. In support of this idea, Chapter 3 indicates that the perceptual judgment of sub-second event timing in the visual scene seems to be instantiated in the strength of adapted responses.

In retrospect, it is hard to imagine that adaptation's undeniable temporal information is ignored by the brain. But before we performed the present experiments, there was no evidence that the brain actually might exploit the available temporal information. This insight could help bridge results from both neuroscience and psychology. Whereas neuronal adaptation has been considered a mechanism without a clear function, time perception has been considered a cognitive function without a clear biological mechanism. Our magnitude coding results in FEF (Chapter 3) offer a unifying principle that resolves both issues.

The power of our adaptation framework can be appreciated through a consideration of the psychophysical literature, which has clearly described the precision and limits of human time perception. Like the perception of other stimulus properties (e.g., orientation or brightness), time can be misperceived in powerful duration illusions (Rose and Summers, 1995; Tse et al., 2004; Morrone et al., 2005; Pariyadath and Eagleman, 2007; Terao et al., 2008; New and Scholl, 2009). These illusions are generally elicited in the visual system by presenting stimuli close in time to an eye movement, by changing the brightness of stimuli, or by changing the identity of stimuli. Importantly, these same manipulations also dramatically alter adaptation at the level of single neurons. The temporal information in neuronal adaptation—conveyed by the size of an adapted sensory responses—systematically changes in accordance with the subjects' perception of time in all of these illusions (see the next section for an illustrated example).

Time perception based on neuronal adaptation also accounts for two other common findings in experimental psychology. Many studies show marked differences in time perception at different timescales, e.g., hundreds of milliseconds vs. tens of seconds (for review, see Mauk and Buonomano, 2004). Such results are used to support the claim that there are different internal clocks referenced as needed at different timescales. But the locations of these clocks in

the brain—generally thought to correspond to specific anatomical areas—are unknown. We think it is not a coincidence that sensory neurons themselves respond in a fundamentally different manner at these same timescales. Thus, the elusiveness of a timekeeping locus is not necessarily surprising; temporal information may be distributed in sensory neurons across modalities and brain regions (Mauk and Buonomano, 2004; Buetti et al., 2010), not in specialized areas.

The magnitude hypothesis of time perception unifies two previously unrelated bodies of work: our biophysical understanding of neuronal adaptation and our rich behavioral understanding of time perception. In addition to accounting for a wide range of perceptual results, the hypothesis also allows us to generate novel predictions. Most prominently, if the duration illusions mentioned above solely depend on adapted sensory responses, then the illusions should not occur at timescales beyond those that cause adaptation (e.g., greater than ~500 milliseconds in the visual system). Also, we expect the strength of duration illusions to vary in accordance with the different properties of adaptation across sensory modalities. Lastly, if we manipulate levels of adaptation in specific sensory regions of the brain (e.g., primary visual cortex), the perception of time should be altered accordingly and only in relation to stimuli of the affected modality. All of these predictions could be directly evaluated by using a similar paradigm to the one we used in Chapter 3 and carrying out neuronal recordings in animals trained to behaviorally report their perception of time.

In summary, the magnitude hypothesis of time perception accounts for the majority of existing psychophysical results, provides a novel perspective on temporal processing, and generates predictions for future researchers looking to uncover physiologically-plausible “clocks” in the brain.

4.4 TEMPORAL STABILITY AND VISION

As discussed in Chapter 3, stable visual perception requires the brain to continuously maintain: 1) the positions of objects, and 2) the amount of time each object is visible, despite interruptions such as eye movements. Neurophysiological findings have provided a compelling explanation of “spatial updating”, the process by which vision remains spatially contiguous (Duhamel et al., 1992; Klier and Angelaki, 2008). Neuronal mechanisms such as visual remapping (Colby and Goldberg, 1999) and predictive sensorimotor commands (Sommer and Wurtz, 2008) currently provide the best explanation for how the visual world remains perceptually stable despite frequent changes in visual input (Melcher and Colby, 2008; Wurtz, 2008).

Far less is known about how the brain counters saccadic interruptions to maintain a stable perception of time, what we term “temporal updating”. Psychologists have studied temporal updating by presenting human subjects with variable-duration stimuli around the time of an eye movement. Subjects are then asked to judge the duration of the stimuli, and these judgments are compared with those made in baseline conditions during stable fixation. These experiments reveal two major effects: compression of perceived time just before an eye movement (Morrone et al., 2005; Terao et al., 2008) and elongation of perceived time just after an eye movement (Yarrow et al., 2001; Yarrow et al., 2004b; Georg and Lappe, 2007). These perceptual fluctuations provide insight into the brain’s attempt to maintain an accurate representation of time across eye movements.

Although saccade-induced time distortions are often interpreted in the context of spatial stability mechanisms, such an explanation is problematic. Using behavioral data to make inferences about neuronal processing, psychophysicists invoke corollary discharge (CD) and

shifting receptive fields to account for their findings (Yarrow et al., 2004b; Morrone et al., 2005). However, spatial updating alone (at least as we currently understand it) cannot account for perisaccadic time distortions. The largest discrepancy between spatial updating mechanisms and perisaccadic time distortions is that time illusions do not appear to scale with saccade size (Morrone et al., 2005; Hunt et al., 2008). This point is critical because the fundamental utility of corollary discharge information is that it provides both spatial and temporal information about an impending saccade (Sommer and Wurtz, 2008). Therefore if corollary discharge influences temporal distortions, larger amplitude saccades—which are inherently longer in duration—should lead to greater distortions, in accordance with the saccade-duration information contained in the CD signal. But this scaling with saccade size does not occur (Morrone et al., 2005; Hunt et al., 2008). Thus, spatial stability mechanisms cannot fully account for the systematic changes in perisaccadic time perception.

In light of our results in Chapter 3, we posit an additional explanation of saccade-induced temporal distortions based on three of our findings. First, and most importantly, our data indicate that single visual neurons can represent brief time intervals by virtue of the size of their second visual responses (Chapter 3). This is a central point for the interpretation of psychophysical findings because distortions of perisaccadic time judgments are often elicited using identical sequential stimuli at adaptation-like timescales (Yarrow et al., 2001; Yarrow et al., 2004a; Morrone et al., 2005; Terao et al., 2008). Second, in one monkey prior to neuronal recordings, we tested for changes in post-saccadic perception of time intervals. In agreement with human psychophysical results, the monkey chose the “long” target more frequently as the first stimulus was presented closer in time to saccade offset (Fig. 32), indicating an elongation of perceived time. That is, the same time intervals presented during control conditions far after the

saccade were uniformly perceived as longer when presented closer in time to (but never during) the end of an eye movement. These preliminary results support the conclusion that monkeys experience the same saccade-induced illusions that humans do, an unsurprising finding given the well-known, close similarities between the monkey and human visual and oculomotor systems.

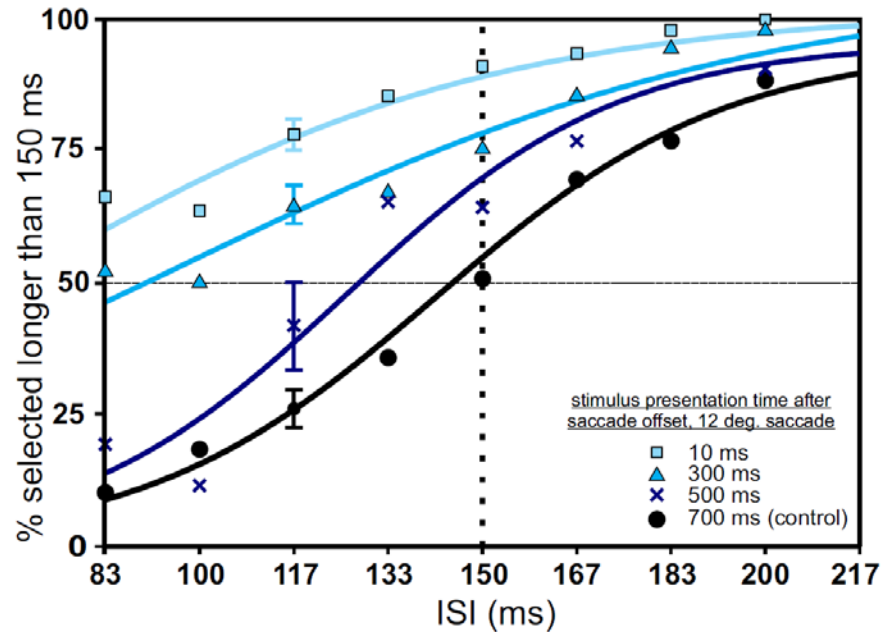


Figure 32. Behavioral evidence of post-saccadic elongation of time perception in monkey K

The reference interval was 150 ms interstimulus interval (ISI; dashed vertical line). As time intervals (x-axis) were presented closer to saccade offset (increasingly lighter-colored symbols), the monkey chose the “long” choice target more frequently. Representative SEMs for the curves are indicated by the error bars at ISI 117 ms.

Third, neuronal responses to stimuli presented around the time of an eye movement are subject to saccadic modulation (Chapter 2; Reppas et al., 2002; Royal et al., 2006; Ibbotson et al., 2008). This modulation, in the form of saccadic suppression (Matin et al., 1972; Matin, 1974), decreases neuronal responsiveness just before, during, and just after an eye movement. In Chapter 2, we illustrated post-saccadic suppression of visual activity in FEF neurons ~30 ms after saccade offset.

These three experimental results (magnitude encoding of perceived time intervals, behavioral evidence of postsaccadic elongation, and postsaccadic suppression of visual activity), taken together, provide a compelling explanation for time illusions associated with eye movements. During periods of fixation, sequential visual responses are adapted and unaffected by peri-saccadic modulation (Fig. 33A). Now let us consider what would happen if presentation of the same two stimuli defining the time interval are moved forward in time, so that the second stimulus is presented just before an eye movement (Fig. 33B). We would expect to see a suppressed visual response to the second visual stimulus due to early effects of saccadic suppression. At the same time, the human psychophysical literature suggests that the perception of time is compressed (Morrone et al., 2005; Terao et al., 2008), or shortened relative to the control condition (Fig. 33A). Our results in Chapter 3 suggest that this compression of perceived time is directly related to the weakening of the second visual response. Finally, two stimuli presented immediately after the saccade results in a suppression of the first visual response. The later, second neuronal response would be unaffected by the saccade, which would decrease the difference between the two visual responses. If we assume that the magnitude hypothesis takes into account some information about the relative strengths in first and second responses, then diminishment of the first response effectively increases the size of the second response. We showed that this presentation scheme elicits relatively more “long” choices in our monkey subject (Fig. 32). The most parsimonious explanation of this time illusion, then, is that the saccade causes changes in the visual responses, and the relative increase in second visual response strength leads to changes in time perception as predicted by the magnitude hypothesis.

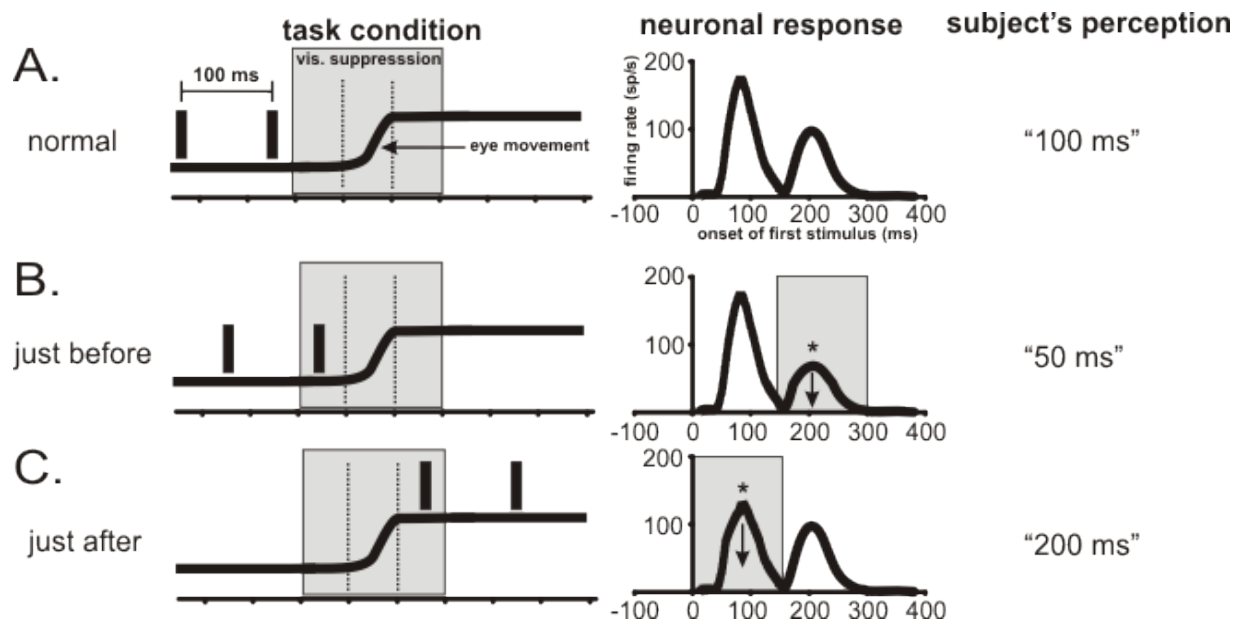


Figure 33. Illustration of proposed neural basis of peri-saccadic time illusions

A, Control condition, where the interval is presented long before the time of the eye movement. B, Just before the eye movement condition. C, Just after the eye movement. In left column: thick black line represent eye position; the beginning and end of the eye movement are bracketed by vertical dotted lines; gray box represents the time of neuronal suppression caused by the eye movement; each tick marks represents 50 ms. In the right column, activity is aligned to the onset of the first visual stimulus at time zero.

Our magnitude hypothesis provides a new basis for generating testable predictions for future experiments. For example, if short time intervals are represented by the difference in the magnitude of sequential neuronal responses, then experimental manipulations of one or both responses should correspondingly alter perception of interval duration. Changing the relative brightness of a stimulus is one way to alter the size of a resultant neuronal response and, as predicted, this manipulation alters perceived duration in human subjects (Terao et al., 2008). However, many questions remain unanswered. What are the limits of time distortion? Can time lost during a saccade be “re-constituted” by using higher contrast stimuli or, alternatively,

electrical microstimulation of neuronal circuits (see above)? If our subjective experience of time can be altered by hundreds of milliseconds, what does this imply about a putative “internal clock” in the brain? These are some of the thought-provoking questions that need to be addressed in future experiments.

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